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NOTICE

For reasons of national paper economy the number of pages in *Biological Reviews* will be reduced during the remainder of the war. We intend nevertheless to publish three articles quarterly; this will be made possible by using smaller type and by reducing the length of contributions. There are only two articles in the present number because these had been set up in the old type before the reduction in our paper quota was announced. The attention of future contributors is drawn to the wartime instructions for the preparation of manuscripts printed inside the back wrapper of the present number.

CONTROL OF MOVEMENTS BY THE CEREBRAL CORTEX IN PRIMATES

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I. INTRODUCTION

Hughlings Jackson in 1869 divided the cerebral cortex into two large divisions separated by the central fissure, an anterior which served 'in the motor aspect of the mind' and a posterior which might well serve 'in the sensory'. Such a generalization becomes extraordinarily acute when it is remembered that Jackson's deduction was made upon clinical material, unaided by surgical intervention or by animal experimentation. That generalization preceded electrical stimulation of the cortex cerebri as well as all cytoarchitectural studies except the four-layered cortex of von Kölliker (1855) and the five layers of Meynert (1868). That 'the motor aspect of the mind' was served by any particular type of cellular structure seemed also to be unknown.

To consider the control of movements by the cerebral cortex 73 years later requires correlation of several different types of data. Now that surgical intervention in cases of tumour or of traumatic epilepsy is the rule, clinical approaches experimental material in value, (1) as the result of electrical stimulation of the surface of the cortex cerebri, and (2) as the result of cortical removals. Although our knowledge is far from complete (cf. Hines, 1929), a great deal has been added recently by the savants of cytoarchitecture (cf. Hines, 1934) and by the students of cortici-fugal and cortici-petal systems. Moreover, in animals, particularly in primates such as the chimpanzee and the macaque, it is possible

to correlate a great variety of data. This review will attempt such a correlation of clinical data with those of animal experimentation and undertake an analysis of the contribution of the cerebral cortex to the control of movement.

II. ORGANIZATION OF THE CEREBRAL CORTEX

Since the time of Fritsch & Hitzig (1870), Betz (1874, 1881) and Bevan Lewis (cf. Lewis & Clarke, 1878), that part of the cortex which controls movements has been allocated to the prerolandic area and associated with a definite cytoarchitecture. This cortex was noted by Brodmann (1903) to lack a definite IVth layer and to possess rather thick IIIrd and Vth layers, each of which was largely composed of pyramidal cells. Some of these pyramidal cells in the Vth layer were unusually large, measuring in man $60–120 \times 20–60\,\mu$ by $80 \times 50\,\mu$ (Economo & Koskinas, 1925, p. 48), the giant pyramids named for Betz. These enormous cells are not evenly sown through the Vth layer of the precentral gyrus (Brodmann's area 4); rather they occur in fairly evenly spaced clusters of five or six cells throughout the inner pyramidal layer (Betz; Lewis & Clarke). The axones of these cells penetrate the spinal cord (Holmes & Page May, 1909) and form part of the tractus cortico-spinalis (other origins, postcentral gyrus, Vogt & Sachs, cf. Foerster, 1923; IIIrd layer of area 4, Spielmeyer, 1906). To demonstrate that this cortici-fugal system was interrupted in paralysis of cortical or capsular origin took years of correlation of clinical and pathological findings (cf. Charcot & Pitres, 1895). The modification of that correlation has been almost equally difficult. Consequently the precentral gyrus became 'the' motor cortex and the pyramidal tract 'the' cortici-fugal system, in spite of the fact that other cortici-fugal systems such as the cortico-pontile (Dejerine, 1901), the cortico-thalamic (Dejerine, 1901; Vogt, cf. Foerster, 1923), the cortico-rubral and cortico-nigral tracts (von Monakow, 1909, 1910) were well known. Indeed, it was not until Foerster (1923, 1931) repeatedly outlined the results of electrical stimulation of the surface of cortical areas outside the precentral gyrus of man that the importance of extrapyramidal motor patterns was recognized. And this was about 50 years after the publication of Ferrier's monograph (1876) on the functions of the brain, in which he recorded positive results of electrical stimulation of the parietal, occipital and temporal lobes and of the frontal lobe outside the region inhabited by the Betz cells. These particular results of electrical stimulation suggest an organization of cortici-fugal systems within each of the separate lobes apart from area 4 itself. What correlation exists between the known cortical projection systems, the results of electrical stimulation and the cytoarchitecture of the cortex cerebri?

1. ARCHITECTONICS OF MOTOR AREAS

The structure of the sheets or layers of cells found within the region which is electrically excitable even under deep ether anaesthesia has become the prototype for motor areas. As indicated above, such areas lack a definite IVth layer and have well-developed IIIrd and Vth layers. In the frontal lobe these areas are known collectively as the area frontalis agranularis, a type of cortex designated by Economo & Koskinas as agranular isocortex. The agranular isocortex of those authors (1925) included not only Brodmann's areas 4 and 6 of the frontal lobe, but also the anterior division of the gyrus cinguli, the subcallosal gyrus, the antero-inferior border of the insula, and the anterior tip of the temporal lobe. Of these areas in man only the area frontalis agranularis has responded to electrical stimulation.

Outside of the cytoarchitecturally motor areas of *Cercopithecus* Ferrier (1876) was able to elicit movements of head and eyes to the opposite side by faradic stimulation of the area frontalis granularis (Brodmann's areas 8 and 9). In the parietal lobes (area 7) and occipital lobes (areas 18 and 19) conjugate deviation of the eyes to the opposite side with either upward or downward deviation and constriction of the pupils were produced. Pricking up the opposite ear, rotation of the head and eyes to the opposite side and dilatation of the pupils was obtained from the superior temporal gyrus (area 22). In the apes Grünbaum & Sherrington (1901) and Leyton & Sherrington (1917) obtained conjugate deviation of the eyes from the second frontal convolution and the area striata. Similarly, stimulation of the area striata (Walker & Weaver, 1940) of the macaque gave conjugate deviation upward, ventral to the calcarine fissure and conjugate deviation downward, dorsal to that fissure. To these results the Vogts (1926) in *Cercopithecus* and Foerster (1931, 1936) in man added the elicitation of deviation of head and trunk to the opposite side (their adversive movements) by stimulation of the superior parietal lobule (area 5) and of the postcentral gyrus.

No common denominator of cytoarchitecture exists among these stimulated areas. However, the regions surrounding the sensory projection areas of the cortex cerebri, or Flechsig's primary areas, are characterized by large pyramidal cells in the IIIrd layer (cf. Economo & Koskinas). Such regions, with the exception of areas 18 and 22 in man, have been proved to be electrically excitable.* Besides these areas and the divisions of the agranular isocortex which have been stimulated there are others, particularly those in the area frontalis granularis, which respond to the electric current and share no architectural features with either one of the excitable regions listed above. On the other hand, electrical excitability may be an expression of subcortical destination of cortici-fugal pathways.

2. Cortici-fugal pathways

For the cortex of man both Foerster (1923; 1936) and Economo & Koskinas (1925, p. 134) have collected the cortici-fugal pathways. The pyramidal pathway or tractus cortico-spinalis is made up of axones of the giant (Betz) and other pyramidal cells in layer V of area 4 (Wohlfahrt, 1932) and perhaps of large pyramidal cells in the lowermost part (*c*) of layer III (Spielmeyer, 1906; Wohlfahrt, 1932). In monkeys (cf. Foerster, 1923) degeneration of the fine fibres of the pyramids was reported to follow removal of the postcentral gyrus (areas 3, 1, 2). The cortico-pontile fibres originate from each of the four lobes (i.e. the orbital and middle frontal gyri, the middle and inferior temporal gyri, the gyrus angularis, the superior parietal lobule, and part of area 19); the cortico-rubral system, from the orbital gyri, the anterior part of the superior and middle frontal gyri and the pre- and postcentral convolutions (von Monakow, 1909; Dejerine, 1901); the cortico-nigral fibres, from the regio Rolandica and the foot of the first frontal gyrus (Flechsig, 1876) and from the opercular region of the pre- and postcentral gyrus and the contiguous regions of the frontal and parietal lobes, as well as the middle part of the temporal lobe (Dejerine, 1897). The cortico-thalamic connexions have been described

* The term 'electrically excitable cortex' is used in the old sense; for it is applied here to areas from which movements are elicited by stimulation of the cortical surface with an electric current. In the strict sense of the term all of the cortex is electrically excitable as the techniques of electrical recording from its surface have shown. In the near future some distinction between these two uses of the term 'electrically excitable cortex' will have to be made.

as reciprocal with the thalamo-cortical fibres (Dejerine, 1901; von Monakow, 1905) and form in man important descending systems from the foot of the middle and inferior frontal gyri, from the medial surface of the frontal lobe including the gyrus cinguli and from the precuneus. Besides these Dejerine has described descending fibres from the occipital lobe (probably area 19) which ended in the superior colliculus and fibres from the first temporal convolution (probably area 22) which terminated in the inferior colliculus. Economo & Koskinas suggest that the large pyramids of the Vth and of the IIIrd (*c*) layers are well developed in the regions from which the cortico-spinal, the cortico-bulbar and the cortico-nigral tracts originate, that the Vth and VIth layers are well developed in the areas from which the cortico-pontile springs and that nerve cells in the VIth layer may be the origin of the cortico-thalamic (Nissl, 1911) and cortico-rubral tracts.

The cortici-fugal pathways now known to exist in the monkey suggest that the list given above for man is incomplete. Besides the older studies of Minkowski (1923, 1924 *a*, *b*) on the macaque, those published since 1935 cover large areas of the cortical surface of the brain in a single species, *Macaca mulatta*. In order to enable the reader to comprehend at a glance the status of this knowledge, I have listed in a chart (Table 1) the origin (by areas or by lobes as given by the author) and the termination (as discrete nuclei where possible) of the cortici-fugal systems which these authors believe to exist.

Cortici-fugal systems fall naturally into two groups, those which end in subcortical sensory nuclei (the dorsal thalamus) and those which terminate in motor nuclei of the brain stem and spinal cord. Comparing the known efferent projection systems of the prerolandic regions with those of the postrolandic mantle it is evident that a greater variety and bulk of the cortici-fugal fibres to motor nuclei are derived from the frontal lobe than from any other single lobe. Within each of the four lobes components of both the cortico-bulbar (i.e. to the medulla oblongata) and cortico-pontile systems originate (Mettler, 1935). Outside the frontal lobe both the parietal and temporal lobes give rise to fibres destined for the nucleus ruber (Mettler, 1935), for the substantia nigra (Mettler, 1935; temporal, Bucy & Klüver, 1940), and for the tegmentum of the medulla oblongata (Mettler, 1935). As should be expected from the results of electrical stimulation of area 7 of the parietal lobe and of areas 18 and 19 of the occipital, cortico-mesencephalic (superior colliculus) fibres were found (Mettler, 1935). From the temporal lobe Marchi degeneration penetrated the inferior colliculus only (Mettler, 1935).

Non-pyramidal efferent fibres from the posterior division of area 4 are similar to those from area 6 (Levin, 1936). Although these non-pyramidal systems were found to have similar destinations their exact termination within that destination had a specific localization. Besides the projection into the lateral thalamic nucleus these fibres ended in the subthalamus, the nucleus ruber, the substantia nigra, the cranial nerve nuclei, the tegmentum (medulla oblongata) and the pons. Verhaart & Kennard (1940) did not find any cortico-rubral system arising in the small pieces which they burned out of the posterior division of area 4, of the anterior division of the arm area (4*s*), or of area 6. Marchi preparations (Hines, unpublished) showed no trace of degeneration into the nucleus ruber or into the nuclei of the cranial nerves after removal of the anterior border of area 4 (i.e. 4*s*). There was, moreover, marked evidence of degeneration into ventral and lateral nuclei of the thalamus, into the septum pellucidum, the gyrus subcallosus

Table 1. *Source of origin and termination of cortici-fugal pathways in* Macaca mulatta. *The capital letters refer to the source of the authority responsible for the description of the different systems (B, Brouwer; B and K, Bucy and Klüver; H, Hines; L, Levin; L and B, Levin and Bradford; M, Mettler; V and K, Verhaart and Kennard) and references can be obtained by consulting the bibliography.*

CORTICI-FUGAL FIBERS to / from	FRONTAL									PARIETAL			TEMPORAL				OCCIPITAL		
	4	4s	6	8	9	pref. a.	mid. g.	inf. g.	total	post-c. g.	5 : 7	total	sup. g.	mid. g.	inf. g.	total	17	18	19
Nucleus caudatus					M				L M										
Globus pallidus									L										
Nuc. thalamicus lat.	L M V&K	H V&K	L V&K		L	L						M	M				M		
Nuc. thalamicus vent.		H				L													
Nuc. thalamicus med.						L								M					
Pulvinar												M		M		M B&K		M	M
Lateral geniculate body																B M		M	M
Medial geniculate body													M			M			
Subthalamus	L V&K	H V&K	L V&K				M	M		M						M			
Hypothalamus	M											M	M						
Septum pellucidum	M	H																	
Gyrus subcallosus		H																	
Superior colliculus	M						M				M							M	M B&K
Inferior colliculus																M			
Tegmentum, midbrain		H				L		L											
Nucleus ruber	L M		L		M		M	M		M						M			
Substantia nigra	L V&K	H V&K	L V&K	L		M						M	B&K	M					
Bulb	L		L				M	M				M				M		M	M
Tegmentum, med. obl.	L M	H	L	L								M				M			
Inferior olive	M											M							
Pons	L V&K	H V&K	L V&K		M	L	L				M			M	M		M	M	M
Spinal cord	L V&K	H								L&B	L&B								

and into the tegmentum of the midbrain.* Mettler's removal of area 4 also found a cortico-septal degeneration and a cortico-hypothalamic and a cortico-mesencephalic (superior colliculus) as well.

Area 8 sends fibres to the tegmentum of the medulla oblongata and to the substantia nigra (Levin); area 9, to the lateral thalamic nucleus (Levin), to the nucleus ruber and to the pontile nuclei (Mettler); the whole prefrontal region, to the lateral, ventral, and medial nuclei of the thalamus, to the tegmentum of the midbrain and to the pons (Levin) as well as to the substantia nigra (Mettler). Both Mettler and Levin thought that removal of the whole frontal lobe resulted in degeneration of fibres in the subcallosal bundle and that some of these fibres penetrated the nucleus caudatus; and Levin considered that such an ablation also caused degeneration of tracts into the globus pallidus.

Levin & Bradford (1937) studied retrograde degeneration of nerve cells in the frontal and parietal lobes following a hemi-section of the cord in the cervical region, and thought that they had proven the tractus cortico-spinalis to originate not only in area 4 but also from the whole of the parietal lobe.†

This striking array of known extrapyramidal systems in the macaque calls for a search of their contribution to the performance of muscle. Will electrical stimulation of the cortical surface from which they arise evoke their activity and, if so, is it possible to separate these results from the movements which have been allocated to the stimulation of the pyramidal tract?

III. ELECTRICAL STIMULATION OF THE CEREBRAL CORTEX

The response of the cerebral cortex to electrical stimulation can be read in the visible or palpable changes elicited in skeletal muscle. These results in turn may be classed as the characteristics of excitability of this suprasegmental mantle and are (1) the types of movements elicited, (2) phenomena such as tonic innervation, inhibition, and relaxation, and (3) the typical distribution of reactive loci. The results of stimulation of any given reactive locus depends not only upon its position on the cortical mantle, but also upon the physiological state of the animal and the strength, frequency, and form of the stimulating current (cf. Clark & Ward, 1941).

1. MOVEMENTS

Two general types of movements have been elicited, one which is dependent upon the intactness of the cortico-spinal or pyramidal tract, and one which is independent of that pathway and known as extrapyramidal.

(*a*) *Results of electrical stimulation of area 4 with the pyramids intact*

In Foerster's last analysis (1936) of the results of stimulation of area 4 of the precentral gyrus in conscious man, he localized the greater part of the lower extremity on the paracentral lobule, thus disagreeing only slightly with Scarff (1940) who reported the whole

* It may be well to record for purposes of completeness that degenerated myelin exactly similar to that found within the brain stem entered the brachium pontis and terminated (?) within the nucleus dentatus and the cerebellar cortex in the region of the Purkinje cells. This was not included in the above list of cortici-fugal pathways from the anterior border of area 4 (4*s*) because it must be substantiated in another macaque before it is granted the degree of certainty necessary for such inclusion.

† Since this review was written T. L. Peele (Amer. Ass. Anat. 58th Ann. Meeting, N.Y.C.) reported that degenerated myelin was found in the region of the lateral cortico-spinal tract contralateral to differential lesions of the parietal lobe of the macaque; extending to the cervical levels after ablation of area 5 or of area 7 and to the lumbar or sacral levels after removal of areas 1 and 2.

of the leg as located on the medial surface. Within the usual somatotopical fields Foerster described separate foci for antagonistic movements of single parts of the body or of parts of an extremity, and special motor elements for each single muscle group and in some instances elements for single muscles or even parts of muscles. At some level (probably cortical) these single elements are in close conjunction with other elements because these single movements appeared co-ordinated when nearby loci were stimulated. Foerster interpreted all movements which he elicited as pyramidal even if some of them involved bilateral contraction of the distal musculature of the extremities. Besides bilateral contraction of muscles of head, face, neck, trunk and proximal joints of the extremities Foerster reported bilateral movements of fingers when very strong currents were used. So far no other neural surgeon has reported comparable results (cf. Penfield & Boedrey, 1937).

In the recent reports of electrical stimulation of the cortices of animals pyramidal types of movements have been elicited outside of area 4. The hind-leg area both of the rabbit (Brooks & Woolsey, 1940) and of the opossum (Bromiley & Brooks, 1940) has been identified outside of area 4. And in the goat (Clark, Ward & Dribben, 1941) only the movements of the extremities were obtained from the sigmoid gyrus.

Stimulating the leg area of the macaque with the 60 c.p.s. sine-wave current in a mosaic of points placed 2 mm. apart antero-posteriorly in rows 1 mm. apart mediolaterally, Woolsey (1938) was able to project upon the cortex a segmental pattern of the musculature of the lower extremity, muscle by muscle. Muscles belonging to the dorsal sheet (see p. 221) were located on the medial surface of the hemisphere, those of the ventral sheet on the dorso-lateral surface.

Using this current, stimulation of the precentral gyri of adult chimpanzees (Hines, 1940) gave results which differed from the classical work of Sherrington (with Grünbaum, 1901; with Leyton, 1917). Different loci for antagonistic movements were found, namely, finger flexion was ventral or anterior to extension, toe extension was ventral to toe flexion, supination and pronation were separated, and extension of the wrist was dorsal to flexion of the wrist. Contractions of isolated muscles or parts of muscles were obtained. The fact that the loci which yielded the contraction of single small muscles often covered larger cortical areas than those from which larger muscles responded suggested that the number of cortici-fugal fibres to certain motor nuclei within the ventral horn are more numerous than those to other nuclei. Certain co-innervations frequently occurred, representing activation of dorso-lateral or ventro-lateral groups of nuclei within the ventral horn of the spinal cord, innervating respectively flexor or extensor sheets of muscles. Other co-innervations suggested patterns of progression or of posture, possible innervation of brain-stem mechanisms, while still different co-innervations of flexor and of extensor muscles hinted that cortically selected patterns had been stimulated. For these patterned movements were the ones which were used in life by the animal as long as the pyramidal tract was intact and could not be evoked either by the animal or by cortical stimulation when that system was surgically interrupted (Tower & Hines, 1942).

(*b*) *Results of stimulation of extrapyramidal motor systems*

(i) *Pyramids intact*

Foerster (1931, 1936) located in man two varieties of extrapyramidal fields: (1) those which yielded turning of the head, trunk, and extremities (plus or minus deviation of the eyes) toward the opposite side, and (2) those which yielded deviation of the eyes only,

when electrically stimulated. The first type of movements was obtained from the posterior part of area 6, the anterior part of area 6, the postcentral gyrus, the anterior part of the superior parietal lobule and the superior temporal gyrus. Foerster's eye fields were distributed over three of the four lobes, area 8 of the frontal lobe, the posterior part of the superior parietal lobule and area 39 (the gyrus angularis), and area 19 of the occipital lobe.

Electrical stimulation of homologous areas in the cortical mantle of animals produced three general types of movements. The first two were similar to those described above for man and the third might add to either of those types or involve solely movement of both ears or of the opposite ear. Two eye fields have been found in the chimpanzee, one about the calcarine fissure of the occipital lobe (Grünbaum & Sherrington, 1901), and one about the middle frontal sulcus (Grünbaum & Sherrington, 1901; Dusser de Barenne, Garol & McCulloch, 1941). In the macaque, Ferrier (1876) outlined three of the four eye fields, neglecting the one in the area striata (cf. Walker & Weaver, 1940). The Vogts (1926) described only one in the frontal lobe (area 9) of *Cercopithecus*; Hines & Straus (unpublished, *Macaca radiata*) found one on the anterior border of area 6 and another in area 8 of the frontal lobe, as well as a third in area 17 of the occipital cortex. For *Macaca mulatta*, Tower & Hines (1942) have observed eye fields, upon each of the four lobes, one on the lateral surface of the frontal lobe (in areas 6, 8, and 9), one on its medial surface (area 6), two in the parietal lobe, i.e. one on the lateral surface in area 7, one on the medial surface in the precuneus region, only one in the occipital lobe (area 19; area 17 was not stimulated in these experiments; cf. Walker & Weaver, 1940) and one in the temporal lobe covering the upper half of the first temporal convolution (area 22). Movements of the opposite or of both ears accompanied the eye movements elicited by stimulation of area 6 (*Macaca radiata* and *M. mulatta*), of area 22, and sometimes of area 19. Ferrier evidently obtained movements of the ear only upon stimulation of the superior temporal gyrus.

In the cat (Tower, 1936) as well as in the goat (Clark *et al.* 1941) eye movements have been elicited from the region anterior to the motor cortex and from the gyrus suprasylvius posterior between the receptive areas for sight, somaesthetic sensibility, and hearing. Pricking of both ears was found ventral to this eye field in the cat. Movement of the contralateral ear in both cats and goats was found anteriorly in this same gyrus. But in the chimpanzee movements of the ears have been obtained only upon stimulation of loci in the anterior part of the precentral gyrus (Hines, 1940; Dusser de Barenne *et al.* 1941).

Adversive movements of the trunk and of the appendages in the macaque have been reported by the Vogts (1926) similar to those described for man by Foerster. Tower & Hines (1942) have found similar movements with deviation of the eyes and sometimes with elevation of the ear not only by electrical stimulation of the frontal lobe but also in the occipital (area 19) and in the temporal (area 22) lobes of *Macaca mulatta*. No adversive movements have been reported as the result of electrical stimulation of extrapyramidal fields in the chimpanzee, although movements of the extremities similar to the synergies of Foerster have been elicited from area 6 (Hines, 1940). Although somatotopical bands were drawn across the central fissure stretching from the postcentral gyrus to the middle of the frontal convolutions by Dusser de Barenne *et al.* (1941), no attempt was made by the writers to correlate these results with those of other workers. And it

would have been difficult. Since the results of stimulation of the frontal convolutions and of the postcentral gyrus duplicated those of the precentral gyrus, it was probable that the efferent pathway used was the cortico-spinal reached via transcortical fibres. Anteriorly in the superior frontal gyrus these workers observed quadrupedal progression and alternating forceful expirations. There is much yet to learn about the cerebral mantle of the chimpanzee.

(ii) *Pyramids surgically divided*

Besides the adversive movements obtained by stimulation of the cortical surface far removed from the field of origin of the pyramidal tract, contraction of skeletal muscle may be elicited by stimulation of that field itself either after surgical division of the medullary pyramids (cf. Mettler & Mettler, 1940) or before the pyramidal tract has reached maturity.

Of the four extrapyramidal motor areas in the cat (Tower, 1936) the one in the frontal lobe was the most elaborate, being composed of three fields. The total pattern elicited from the field around the cruciate sulcus was a deliberately developing, deliberately executed diagonal progression or 'pleasure reaction'; that from the field anterior to this region, a suddenly appearing, rapidly executed diagonal progression or striking or placing. The cat ran, tail active, back humped and respiration accelerated. The medial field did not yield alternating movements, rather tonic flexor synergies of the ipsilateral legs (or leg) and tonic extensor synergies of the contralateral legs (or leg).

The parietal extrapyramidal motor area (about fissura ansanata) was the least predictable of these four fields, producing often only adversive movements. The lateral area (inferior division of the anterior limb of the ectosylvian gyrus) gave a synergic flexion of the crossed leg in pronation (claws out) which might reverse and become rhythmic, accompanied by active movements of the tail and by an accelerated respiration. The posterior extrapyramidal motor area (posterior limb of the suprasylvian gyrus) yielded placing of or striking movements of the crossed foreleg, synergic flexion in pronation or in supination of the ipsilateral leg, and diagonal progression.

The cortical extrapyramidal activity elicited by electrical stimulation of the cortical mantle was very nearly equivalent to the total activity of the decorticated cat. Consequently Tower concluded that stimulation of extrapyramidal pathways in this animal elicited patterns of activity determined entirely by the integration of subcortical centres. Cortical selection exercised only the choice of given patterns, which once selected were never modified. There is, therefore, in the cat an extrapyramidal projection system related to each of the sensory projection areas. Of these the parietal field was the least developed and the frontal the most.

The extrapyramidal projection field in the monkey (Tower & Hines, 1942) bounded the posterior division of area 4, extending anteriorly from area 4*s* to cover all of area 6, including area 8*s* (cf. Walker, 1940) and perhaps a part of area 9 and posteriorly from the central fissure to the intraparietal fissure, over the crest and on the medial surface of each of these areas. It also included the borderlands of junction of the three posterior lobes. Stimulation of these areas with the 60 c.p.s. sine-wave current under light ether anaesthesia caused movements and inhibitory action. The elicited movements varied from simple synergies affecting a single extremity to complex movements often with purposive quality involving the total skeletal muscle. The simple flexor synergy was

elaborated to end in grasping or to form a complex reaching and grasping act. The extensor synergy was sometimes elaborated into a complex movement having definite diagonal organization and occasionally into progression. Orienting movements were either adversive or frontal. The former varied from simple movements of eyes or ears, of head or trunk, to complicated ones involving the tail and extremities as well as the axis. The frontal movements brought the face, eyes, or ears from a deviated position toward the mid-frontal plane and suggested the state of being on the alert (cf. Bucy, 1933).

2. PHENOMENA OTHER THAN MOVEMENT

In the cat (Tower, 1936) no tonic innervation separated from movement was observed. Both steady tonic extensions and tonic flexions were produced by stimulation of the medial surface of the frontal extrapyramidal fields. Relaxation of spontaneous or reflexly induced tonic contraction was elicited from an area co-extensive with the pericruciate areas of the cat's frontal lobe, where pyramidal and extrapyramidal motor activity were located.

Inhibition of movement (either spontaneous or reflexly evoked) was obtained from three different cortical fields in the cat—a frontal, a temporal, and an occipital. This inhibition affected movement either by arrest or by quieting. Arrest was characterized by eyes front, head front, and body in attention, and was elicited from the frontal and temporal fields. Quieting was general and was elicited from all three cortical fields.

In the monkey electrically initiated inhibitory action caused (1) relaxation of tonic innervation of skeletal muscle and (2) the cessation of spontaneous movement. With the pyramids intact a topical inhibition of tonic innervation was elicited by stimulation throughout area 4, which disappeared after the pyramidal tracts were severed. Thereafter inhibition was still effective, but most strongly forward in area 4 and in area 6, and was effective bilaterally with no topographical localization. Cessation of spontaneous movement was a general affect and when produced gave the animal 'a curious appearance of attentive repose'.

The cortical fields from which these results were obtained had no clear-cut boundaries. Nevertheless, a certain extrapyramidal organization seemed to exist, for movements and inhibitory action occupied a similar cortical area. Stimulation of the anterior division of area 4 and the posterior part of area 6 yielded synergic movements of flexors or of extensors and diagonal movements; of the anterior division of area 4, inhibition of standing tone. Stimulation of area 6 gave flexor synergies with grasping and inhibition of flexor tone; of areas 6 and 8, and of the temporal and occipital lobes, the reaching, grasping act. Reinforcement of spontaneous tone was obtained from these three areas of the frontal lobe. Orienting movements of the adversive type were separated into two large fields, an anterior which included areas 6 and 8, and a posterior which spread over the junctional region of the parietal and occipital lobes with the superior gyrus of the temporal lobes. Frontal movements were obtained from area 6. Quieting or the inhibition of tonic innervation was a generalized phenomena, being elicited from many regions but most easily from area 9 laterally, and the junctional zones of the three posterior lobes.

Each large division of the monkey's cortical surface therefore had its own adversive field and its own quieting affect. The reaching, grasping act obtained from the occipital

and temporal areas might be associated respectively with sight and hearing. Within the frontal lobe, however, unlike the cat, the extrapyramidal fields have migrated from the pyramidal areas and developed a certain independence with their own movements and their own inhibitions which suffice for the whole cortex.

3. Maturation of 'excitability' in the infant macaque

Anticipating that cortici-fugal systems subserving movement and inhibitory action might develop differentially, the maturation of excitability of the precentral gyrus in the infant macaque was studied (Hines & Boynton, 1940). In this study two general types of movements were elicited (Figs. 1, 2), those which were contralateral and more or less discrete, showing a definite topographical projection pattern upon the precentral gyrus, and those which were not. The first group of movements was called idiokinetic and was dependent upon the intactness of the pyramidal tract. The second group was not discrete, was frequently bilateral, showed no topographical pattern, and was called holokinetic. Although many of them were definitely extrapyramidal, it was impossible to prove all of them to be independent of the pyramidal system.

(*a*) *Holokinetic movements*

These formed a complex group as follows: (1) patterns of progression, (2) movements of girdle and neck musculature, (3) rhythmical movements of lips and of tongue and of the upper extremity, and (4) behaviour patterns. Patterns of progression were elicited from the precentral gyrus of foetal and of infant macaques and from the former after cutting both pyramids. Movements of girdle and of neck musculature were produced by stimulation of the face area of definitive area 4 in foetuses before any sign of pyramidal activity was present (60–125 days gestation). Rhythmical movements of lips and of tongue were obtained from the same area in older foetuses (135–161 days gestation) and in newborns; and rhythmical movements of the upper extremity from the anterior border of the arm area in newborns at a time in development when these areas rarely yielded any idiokinesis to electrical exploration. Two behaviour patterns were elicited, one from the leg area (infantile defaecation pattern) and one from the face area (nursing) only during the time when they were dominant in the infant monkey's activity.

(*b*) *Idiokinesis*

There was a period in the early postnatal development of the monkey during which the elicited idiokinetic movements, although showing a definite topographical projection pattern upon the precentral gyrus, were lacking in discreteness. Rather they were complex co-innervations resembling those executed in life at the age when they were obtained. Strangely enough during this particular period of development the isolated movements which could be elicited before birth (135–161 days gestation) were not again obtained until much later in postnatal development. And in a similar way during the first two weeks of life isolated or discrete movements were occasionally produced electrically from the cortex, movements which were never observed as a part of the use patterns of the infant macaque. Apparently, during the last month of gestation and the first two weeks of life the electric current was able to elicit movements which the animal itself could not execute. After this time the electrically evoked movements corresponded astonishingly well to the movements made by the infant itself.

(c) Phenomena other than movement

Such phenomena elicited by the electric current resembled those already tabulated for the adult, with one exception, that of:

(i) *Fixation*

Visible fixation of muscles proximal to the ones which moved was obtained during the period of development when fixation was visible in the animal's use of its muscles. The

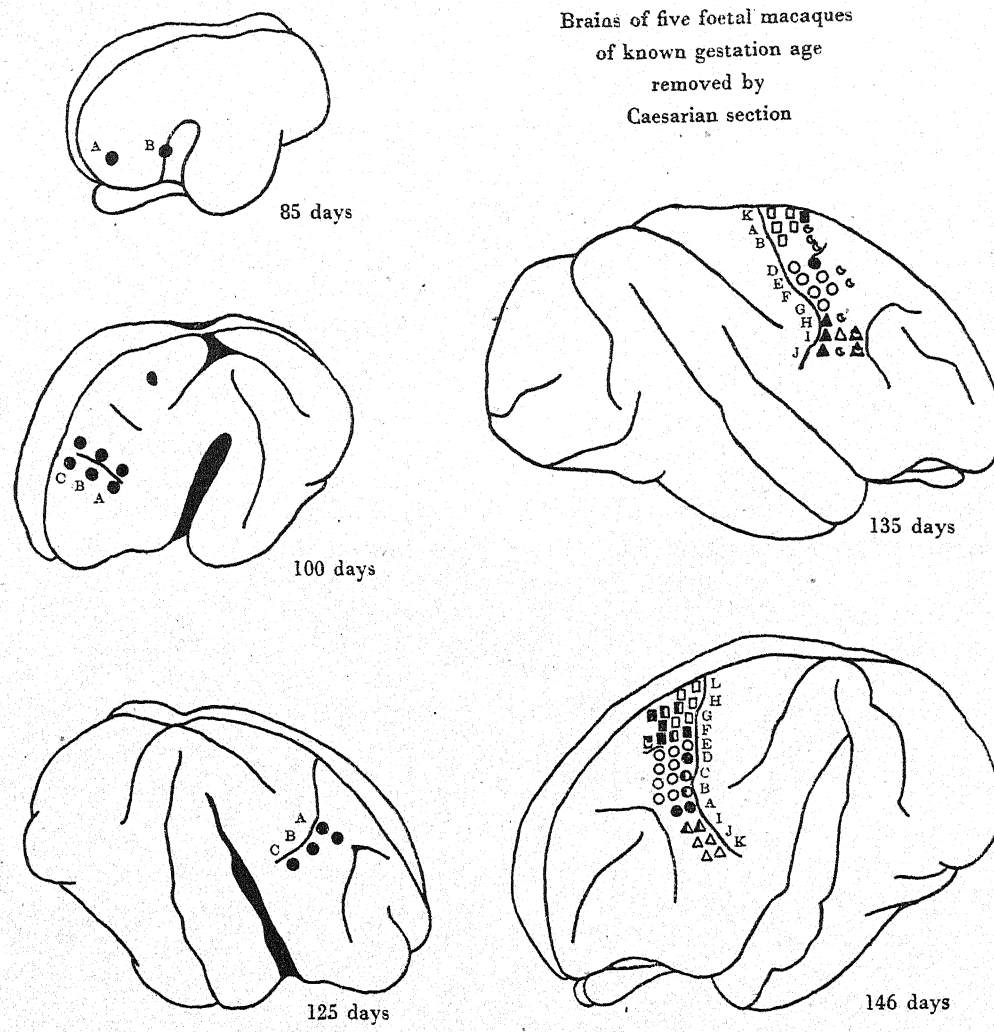

Fig. 1. Development, in the foetal macaque, of parts of the precentral gyrus from which the sine-wave current was able to elicit movements of skeletal muscle and to affect either respiration or tone within striated muscle. The key to the symbols is given in Fig. 2 (from Hines & Boynton, 1940).

particular loci from which fixation of proximal muscles was obtained were the loci which earlier in life had given contraction of those muscles which now became fixed. Indeed, during the first few months of life all idiokinetic movements of distal joints performed by the infant were preceded or accompanied by movement of more proximally placed segments, i.e. the muscle contracted visibly and then became fixed (cf. Beevor, 1903).

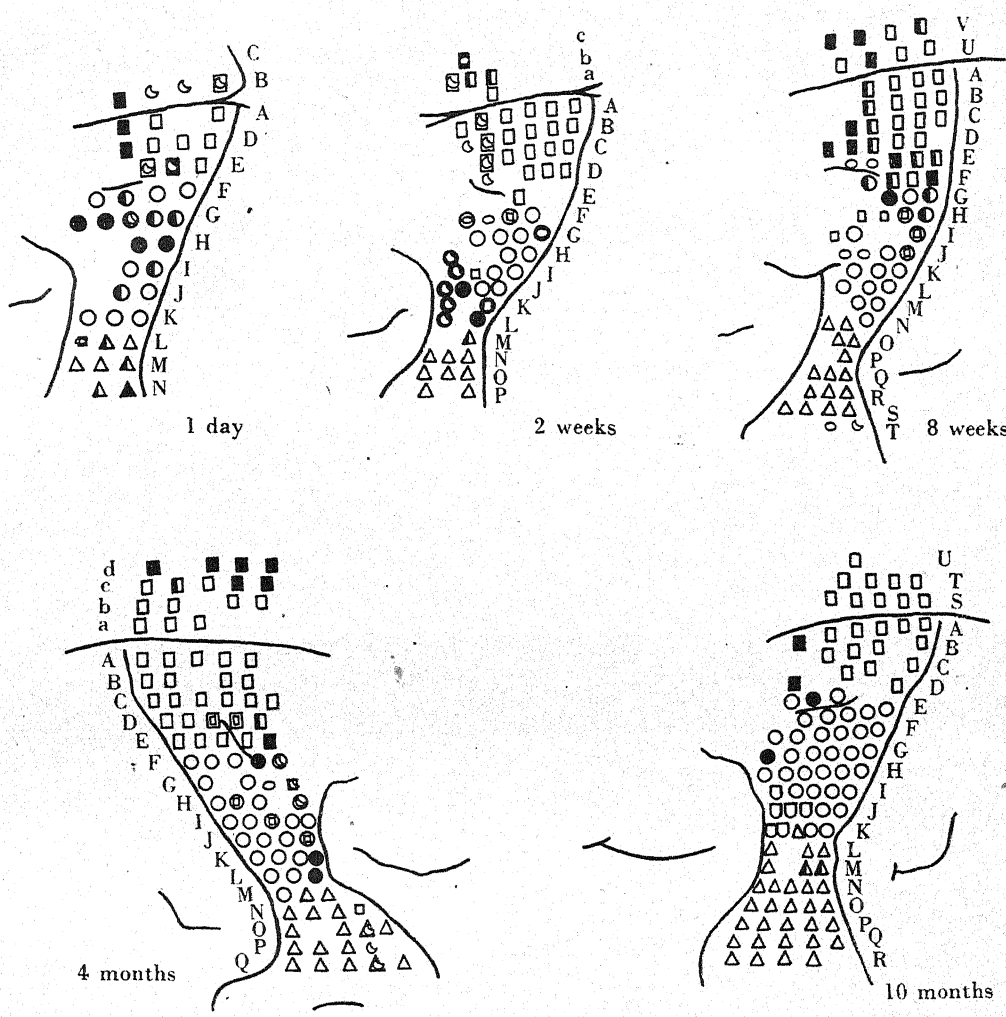

Fig. 2. Development, in the infant macaque, of parts of the precentral gyrus from which the sine-wave current was able to elicit movements of skeletal muscle and to affect either respiration or tone within striated muscle. The key to the symbols is appended to the figure and is self-explanatory (from Hines & Boynton, 1940).

Tonic innervations were frequently and easily elicited from those regions of the precentral gyrus at the time when idiokinesis in that area was in abeyance. Thus in the late foetal period and in the first postnatal week tonic innervations might be obtained from the posterior division of the precentral gyrus, and then on the anterior division, the interregional border of the three main topographical areas and on the ventral or dorsal border of the gyrus itself. Later the locus was confined to the anterior division of the precentral gyrus and then to the anterior border itself.

(ii) *Inhibition of tone or relaxation of tonic contraction*

This was distributed one month before birth and four months after birth within definitive area 4 itself and upon its anterior border. Later inhibition of tone in the intact animal was more easily obtained from the anterior boundary of area 4 and from area 6. Relaxation of tonic extension was obtained before birth from the anterior border of area 4, but no relaxation of tonic flexion has ever been observed before regression of the grasp reflex had taken place.

(iii) *Quieting*

This was elicited by stimulation of the prefrontal areas and of the precentral gyrus as early as one month before birth. As the infant matured after birth, inhibition of movement, although it could be elicited by stimulation of the anterior border of area 4, was more easily produced by stimulation of Brodmann's area 9 of the frontal lobe.

Therefore all of the phenomena which have been obtained in the adult macaque by stimulation of area 4 after surgical division of the pyramids were evoked during some stage of development, in particular when the maturation of the pyramids was incomplete. Apparently then in the intact adult monkey electrical stimulation of the surface of area 4 is more easily transmitted to the cells of origin of the pyramidal tract than to cells of origin of extrapyramidal systems. Further, there is a close correspondence between the motor performance of the infant macaque and the movements which are obtained by electrical stimulation of the precentral gyrus.

4. CONDITIONS OF EXCITATION OF CORTICI-FUGAL PATHWAYS

With or without the surgical division of the medullary pyramids the extrapyramidal systems were susceptible to shock, to interference in cerebral circulation and to anaesthesia. Extrapyramidal movements unlike pyramidal movements were not elicited except under very light anaesthesia. Inhibition of tone was not as susceptible to shock or to interference with the cerebral circulation and was elicited in the pseudo-decerebrate stage (Tower, 1936). The pyramidal tracts, on the other hand, were stimulated under deep ether anaesthesia, under morphine, after slight shock and even after variation in the circulation of the cortex cerebri (cat and adult monkey). Similarly, in the infant monkey with pyramids intact, extrapyramidal activity was obtained only under light anaesthesia and when there had been no difficulty with the circulation of the cortex. Further, in the young infant macaque holokinesis was elicited from an idiokinetic point when stimulated with frequencies of the sine-wave current either lower or higher than the optimum frequency for that particular age. These movements were also more easily obtained from the precentral gyrus before the maturation of the pyramidal unit. Moreover, inhibition of tone did not require as light an anaesthesia as elicitation of holokinesis,

although the anaesthesia must be light enough for the appearance of tone. Therefore the conditions under which idiokinesis, holokinesis and other phenomena are evoked by electrical stimulation of the cerebral cortex of the adult macaque with the pyramids cut are similar to those found by similar experiments upon the precentral gyrus of the infant macaque in which the pyramidal tract is immature.

The intensity of the electrical stimulus was found to be less for the inhibition of tonic innervation than for pyramidal movements and less for pyramidal movements than for extrapyramidal movements in both the cat (Tower, 1936) and the monkey (Tower & Hines, 1942). The gradation of intensity of the stimulating current was similar for the elicitation of these three phenomena in the infant macaque (Hines & Boynton, 1940).

To sum up, the pyramidal tract seemed to be projected upon the cortex of the precentral gyrus as if its origin had a definite segmental arrangement, but no such definite topographical localization was found either for holokinesis in the infant or for extrapyramidal movements in the adult, or for inhibition, whether of tone or of movement in either the infant or the adult.

IV. RESULTS OF REMOVALS OF CYTOARCHITECTURALLY DISTINCT AREAS OF THE CEREBRAL CORTEX

1. Occipital and temporal lobes

After complete removal of either the occipital (Klüver, 1940) or temporal lobes (Klüver & Bucy, 1939) no effect upon the control of movement was reported. The writer found that bilateral occipital lobectomy produced an animal in which all activity was minimal, but that there was no change in movements made, or in the tone of skeletal muscle or in tendon reflexes.

2. Parietal lobes

However, after ablation of the parietal lobe Kennard & Kessler (1940) concluded that fine movements of the distal segments were inaccurate unless corrected by close visual attention, and although the movements themselves were quick and the fingers were capable of individual discrete movements, such movements nevertheless were poorly adjusted in both range and direction. Emotional disturbance appeared to increase the difficulty of the execution of fine movements. Associated movements were not observed. Immediately after the operation the tone in all muscles opposite the lesion was markedly diminished, a diminution which decreased with time but never again approached the normal tone. The knee jerks were depressed at first but later were found to be 'more active' and 'in some cases appeared to be increased'. In my experience the tendon reflexes were never brisk, rather this operation produced tendon reflexes, the excursion of which was greater than found in the normal animal. Atrophy of muscles was reported by Kennard & Kessler in one animal but not found in another with a similar ablation. As might be anticipated the results of differential ablation of this lobe varied; that of the postcentral gyrus resembled that of the total removal of the parietal cortex, while ablation of area 5 was less severe and that of area 7 the least.

3. FRONTAL LOBE

Instead of using the results of removal of the 'motor' cortex, i.e. of area 4 as the standard for projection of the sequelae of ablation of other cytoarchitecturally discrete regions of the frontal lobe, the results of bilateral removal of the frontal lobe will be used.

(*a*) *Bilateral removal of the frontal lobe*

Even with sparing of the face areas this produced an animal which could not sit without support, which could not stand alone, and which was unable to progress in any plane (Hines, unpublished). Food was never swallowed unless it was placed by the observer in the back of the mouth. Indeed, neither objects nor sounds seemed to be recognized and the desire to eat appeared to have perished. The normal distribution of tone in muscles had vanished; for tone was greatly decreased in the extensors of the trunk and in the adductors of the scapula, and greatly increased in the flexors of the trunk and in the elevators of the shoulder girdle. In all of the muscles of the extremities which could be reached by palpation tone had increased. In the upper extremity tone was greater in the flexors than in the extensors, in the retractors than the protractors. In the lower extremity the tone was greater in the protractors and adductors of the thigh, in the extensors than in the flexors of the knee, in the flexors than in the extensors of the ankle. All the muscles of the tail were hypertonic, but only the ventral ones of the trunk.

The resistance to passive movement made by any of these hypertonic muscles was neither 'steady' nor clasp-knife, but approached rigidity, for, unless the trunk was held by the observer, passive retraction of the thigh caused the animal to be brought into the sitting position even before the legs had begun to move. The flexors of the left wrist were so shortened and immobile that passive dorsiflexion of that joint was impossible.

No real relaxation of the hypertonicity was observed during the two months the animal was allowed to live. Even in sleep only a certain quietness was produced, which in no way changed the degree or the distribution of tone.

(*b*) *Bilateral removal of the prefrontal cortex* (*area frontalis granularis*)

This produced a restless, hyperactive animal (Richter & Hines, 1938) without any sign of paralysis, without increase in tone of skeletal muscle and without change in tendon reflexes. There was no increase in resistance to passive movement and no shortening of muscle length.

(*c*) *Bilateral removal of the area frontalis agranularis*

As a contrast to the bilateral removal of the prefrontal area the bilateral ablation of area frontalis agranularis (areas 4 and 6) was followed by paralysis, changes in tone of skeletal muscle, changes in tendon reflexes, and shortening of muscle length. Such an animal was able to progress, to stand upon four feet and even two, and to sit alone. All movement, however, was difficult to start and difficult to stop. Readjustment in movement toward an object was never made. Rather, the initial movement was completed, and if unsatisfactory another movement was made, and so on until the desired object was reached. The more rapid the movement the more accurate the orientation. Emotional excitement increased the ease of the movement but not invariably its accuracy. All movement of the extremities was initiated by contraction of the proximal muscles of the extremity, never by contraction of distal muscles.

Should the animal be completely relaxed resistance to passive movement was greater distally than proximally. Under these conditions the degree of tone became less in extensors and flexors of knee and of elbow, in retractors of the upper arm, and in protractors and adductors of the thigh; but at the wrist resistance to movement remained great in the flexors (ventral and ulnar) and in the pronators, and at the ankle in the ventri-flexors and the invertors.

Tone as determined by palpation was greater than normal in all the muscles of the trunk and tail, except the three rhomboids and the longissimus dorsi. Of these hypertonic muscles the trapezius and latissimus dorsi were shortened. In the upper extremity tone was increased in mm. biceps brachii, brachialis, flexores carpi ulnaris et radialis, flexores digitorum sublimis et profundus and brachioradialis. All of these were shortened except m. brachialis and m. flexor carpi radialis. Less relation between the distribution of hypertonicity and that of shortening was found in the muscles of the leg. Mm. gastrocnemius et soleus, m. adductor longus, and m. tibialis anterior were hypertonic and shortened; m. quadriceps femoris was hypertonic but not shortened, the hamstrings were definitely both hypotonic and shortened.

The degree of relaxation present also modified the quality of two tendon reflexes, the quadriceps femoris and the biceps brachii; for under conditions of general relaxation the reflexly elicited movement was loose and easy and the excursion good. The remaining tendon reflexes were not so definitely modified by conditions of relaxation, for even under the best conditions they were slightly brisk. The triceps brachii recruited its maximum contraction slowly, and only the gastrocnemius irradiated.

Therefore the results of separate ablations of the granular and agranular frontal areas do not equal those of their simultaneous removal. Evidently the granular frontal cortex is able to initiate patterns of progression and to prevent the development of rigidity, and yet when removed separately produces only a hyperactive animal. Similarly the results of differential ablation of parts of the area frontalis agranularis did not equal those of complete removal of the area itself.

(i) *Ablation of the anterior part of the area frontalis agranularis*, i.e. Brodmann's area 6

Richter & Hines (1934) found that this was not followed by any visible sign of paralysis, nor by any change in tone or in its distribution. The tendon reflexes were neither brisk nor quick, neither did they irradiate. No abnormal clonus was present. This operation, however, produced reflex grasping in the hands and feet of the opposite extremity, better developed and more permanent in the hands than in the feet.

(ii) *Ablations of area* 4 *only* (sparing the face)

Hines (unpublished) found that such operations were followed by a differential distribution of paralysis and of tone, by brisk tendon reflexes, by shortening and wasting of muscle, and by the appearance of associated movements contralateral to the lesion.

This paralysis affected discrete and isolated contraction of certain proximal muscles and of all muscles of the digits. Isolated contraction of the protractors, elevators and adductors of the upper arm, of the retractors, external rotators and adductors of the thigh were just as impossible as isolated flexion, extension, abduction or adduction of the digits. Neither isolated supination or pronation of the forearm, nor ulnar or radial flexion of the wrist was observed. Similarly at the ankle no discrete contractions of the

dorsoflexors nor evertors were seen. On the other hand, some discrete flexion and extension of both the elbow and the knee, some discrete retraction and abduction of the upper arm and some protraction and abduction of the thigh occurred. In bilateral lesions the axial extensors were severely paralysed.

Abnormal tone was differentially distributed in the muscles of the contralateral extremities after unilateral lesions and in those of the trunk as well after bilateral lesions. A tonic increment proved permanent in the protractors, adductors, and internal rotators of the thigh, the extensors of the knee, ventri-flexors and invertors of the ankle, retractors of the upper arm, flexors of the elbow, flexors and ulnar flexors of the wrist, and flexors of the fingers opposite the lesion and frequently transient on the side of the lesion. Complete supination or pronation was also impossible in the 'paralysed' arm. And in bilateral lesions increase in tone was found in m. trapezius, m. deltoideus and in the flexors of the trunk. Conversely, a marked diminution of tone was observed in the flexors of the contralateral knee in both unilateral and bilateral lesions, in the extensors of the trunk and in the adductors of the vertebral border of the scapula after bilateral lesions.

The resistance to passive movement offered by these hypertonic muscles was 'clasp-knife' only in the flexors of the elbow and in the extensors of the knee always contralateral and frequently ipsilateral to the lesion.

Brisk irradiating tendon reflexes were always elicited in the contralateral extremities and sometimes in the ipsilateral extremities. However, the tendon reflex of the toes was never elicited in the sitting position and that of m. triceps brachii was rarely brisk. Irradiation more frequently accompanied the elicitation of the tendon reflexes of the ventriflexors of the ankle and the flexors of the fingers than any other tendon reflex studied. Clonus frequently appeared in the ventriflexors or dorsoflexors of the ankle and in the flexors of the fingers.

Shortening of the length to which a muscle can be passively stretched was found in the adductors of the thigh, the flexors of the knee and of the ankle, and in the flexors of the elbow, wrist, and fingers. Certain isolated muscles seem to be selectively shortened, such as the trapezius, latissimus dorsi, brachioradialis, flexor carpi ulnaris, and tibialis anterior. Comparing the distribution of abnormal tone with that of shortening, we find that all shortened muscles with the exception of the hamstrings were also hypertonic. They were hypotonic. But on the other hand, all hypertonic muscles were not shortened (cf. m. quadriceps femoris).

Wasting was characteristic of all skeletal muscles opposite the lesion. Lippett (1942) found that wasting was greater in the muscles of the shoulder girdle than in those of the hip, that the extensors and flexors of the elbow and knee suffered alike, but that in the adductors of the thigh was great (20%). In the muscles of the forearm and the extrinsic muscles of the hand the extensors showed the greater loss, while in the homologous group in the leg the greater loss was confined to three flexors, the gastrocnemius, soleus, and plantaris. Besides these three muscles a marked loss of weight occurred in the coracobrachialis, flexor carpi ulnaris, brachioradialis, the long abductors and extensors of the pollex and in the vastus lateralis. The supinator and the two pronators were noticeably absent from this list.

To sum up, the flexor carpi ulnaris, brachioradialis and gastrocnemius showed maximum wasting, shortening, increased resistance to passive movement and brisk tendon reflexes. The flexors of the knee showed wasting, shortening, and brisk tendon

reflexes with decreased resistance to passive movement. Wasting was negligible in three of the four divisions of the quadriceps femoris. Palpable adaptation reactions were always present in the vastus medialis in which wasting was negligible, while vastus lateralis in which wasting was appreciable never give any palpable adaptation reactions.

Therefore it is evident that wasting, shortening and abnormal tone have different distributions. Wasting, shortening and hypertonus may occur together, wasting, shortening and hypotonus together, shortening and hypertonus without appreciable wasting, wasting alone, and hypertonus alone.

Associated movements were observed in unilateral ablations. For example, yawning was accompanied by protraction and flexion of the 'paralysed' arm; extension of the head by protraction only; scratching, by rhythmic movements of the opposite 'paralysed' arm or leg (cf. Kennard (1940), for results of such ablations on infant monkeys).

(iii) *Differential ablation of the anterior and of the posterior divisions of area* 4

Following ablation of the anterior border of area 4, paralysis was minimal and spasticity maximal; following ablation of the posterior part of area 4, paralysis was maximal and spasticity minimal. Thus a distinction in dominance between two categories of sequelae to total area 4 removals was established.

(α) *After ablation of the anterior border of area* 4 (Hines, 1937), the permanent residual paralysis was confined to the opposite adductors of the thigh and the abductors of the toes. Discrete movements of the distal segments of the hand, including opposition of the thumb and index finger, were never lost. A differential muscular hypertonus was present. In the extremities opposite the lesion tone was maximal in the flexors of the elbow, the ventral and ulnar flexors of the wrist, the retractors of the upper arm, in the extensors of the knee, the ventriflexors and invertors of the foot, and in the protractors and adductors of the thigh. The 'clasp-knife' quality of hypertonus was easily demonstrated as resistance to passive stretching of the quadriceps femoris during the middle 30–40 degrees of the 90 degrees of flexion. During the arc of resistance adaptation reactions were palpable in the vastus medialis.

All tendon reflexes examined in the limbs opposite the lesion were brisk. Irradiation to more proximal muscles accompanied the tendon reflex of the flexors of the ankle, fingers and toes. The extensor of the knee recruited the opposite adductors of the thigh. Clonus was elicited in the contralateral quadriceps femoris, gastrocnemius-soleus, adductors of the thigh, extensors (dorsoflexors) of the ankle, and flexors of the toes. Clonus in the arm was observed in the triceps brachii (rare), in the biceps brachii, and in the flexors of the wrist and of the fingers.

(β) *Removal of the posterior division of area* 4 (Hines, 1937), on the other hand, produced appreciable paralysis and only slight changes in distribution of tone. Tone was decreased in all muscles which attach the extremities to the girdles, slightly increased in the extensors of the knee and flexors of the elbow after months (bilateral lesions), greatly increased in the flexors of the ankle and the wrist, and unchanged in the pronators of the forearm or in the invertors of the foot. All tendon reflexes, except that of the contralateral knee, were not brisk and did not irradiate. No clonus was observed. Paralysis overtook the discrete use of certain muscles: proximally in the retractors or adductors of the contralateral extremities; distally the muscles of the digits, for the fingers and the toes flexed as a group.

Moreover, when the ablation was confined to the arm or the leg area either of the anterior or of the posterior division of the precentral gyrus, the subsequent paralysis was limited to the particular extremity represented in the area of cortex removed. The phenomena of release, however, were not so topically located. Rather they were present in both contralateral extremities. Their quality, however, depended upon whether the lesion was in the anterior or in the posterior division of area 4. Removal of the face area was not followed by any signs of paralysis in the extremities. In this instance the phenomena of release endured in both contralateral extremities for 7 days.

(*d*) *Combined ablation of parts of the area frontalis agranularis*

(i) *Removal of the anterior border of area* 4 *and all of area* 6

This operation (Hines, 1937), performed either simultaneously or successively, produced in time similar residual results. The paralysis had the quality and distribution of the lesion of the anterior border of area 4. The grasp reflex together with brisk irradiating tendon reflexes appeared and then gradually disappeared. Clonus endured as long as one year. Distal to the 2nd joint the hypertonus had the quality and distribution of area 4 lesions; but proximal to that joint the hypertonus was distributed in both the extensors and flexors and its quality was changed. The clasp-knife quality of resistance to passive movement characteristic of area 4 lesions was replaced by a steady resistance, similar throughout the arc of excursion. The most startling sequela to bilateral lesions was the long-enduring hypertonus of the extensors in all extremities (also present but less marked contralateral to unilateral lesions), so great that the assumption of the sitting posture was difficult and for some animals impossible during the first few days or even weeks after the second operation. Even after sitting had become possible the positive supporting reaction remained markedly exaggerated.

(ii) *Ablation of area* 6 *and the posterior border of area* 4

The differential distribution of the hypertonus can be changed by another combination of lesions, namely, that of area 6 and of the posterior border of area 4 (Hines, unpublished). The increase of resistance to passive movement was found in the extensors of the elbow and in the flexors of the knee, and adaptation reactions were palpable in these muscles when stretched. Maximal briskness of tendon reflexes was located in the triceps brachii rather than in the biceps brachii, in the hamstrings rather than in the quadriceps femoris (opposite the lesion). Nevertheless, this operation did not change the distribution of resistance to passive movement which was characteristic of either more proximal or more distal muscles subsequent to lesions of area 4. Like the area 4 preparation the tendon reflex of the flexors of the toes was absent, and unlike that preparation the tendon reflex of m. tibialis anterior was not present (contralateral to the lesion). The subsequent paralysis was similar to that found after ablation of the posterior border of area 4.

4. SURGICAL DIVISION OF THE PYRAMIDS

Tower (1940) found that this affected all somatic muscle below the lesion. This effect was also differential in its distribution, for the decrease in tone was greater in the proximal muscles and the paralysis more serious in the distal muscles. Consequently fixation was gravely affected and discrete movements of the digits were lost. The tendon reflexes were

slow, free and pendular. Unchecked by contraction of their antagonists, unattended by active rebound or after discharge, these reflexes resembled those which characterize lesion of the cerebellum.

Indeed, this generalized tonic deficit may have increased the paralysis produced by pyramidal lesion by raising the threshold for innervation of the extrapyramidal systems which use the segmental mechanism within the spinal cord. The bilateral area 4 preparation has less difficulty with innervation of its somatic musculature than does the bilateral pyramidal animal with that of its musculature below the lesion. Therefore the determination of the degree of muscular incapacity due to paralysis and that due to change in tonic innervation is exceedingly difficult.

On the other hand, certain muscles showed similar paralysis subsequent to lesion of the pyramidal tract, whether that be accomplished at the cortical or at the medullary levels. These muscles were as follows: elevators, protractors and external rotators of the upper arm and the supinator of the forearm; retractors, external rotators and adductors of the hip, the dorsiflexors and evertors of the foot. The greatest loss was found in the discrete movements of the digits, especially the co-operative opposition of the 1st and 2nd digits, the extension and abduction of the fingers, the flexion and abduction of the toes. Besides, the easy initiation of movement, accurate aim and the modification of movement already initiated was lost in the bilateral pyramidal animal, gravely affected in the bilateral area 4 preparation. In both, co-operative movements were slow and weak; their threshold was raised but their basic pattern remained unaltered. Further, the variety of associated movements observed after unilateral pyramidal lesions was greater than after unilateral ablation of area 4.

Muscular atrophy began to be noticeable in the unilateral pyramidal animal after the 4th or 5th month. No contracture or shortening was ever present. The most severe wasting was found in m. rectus femoris and m. brachioradialis. Histologically, no change in the striations was found, no increase in the number of muscle nuclei, and no proliferation of connective tissue. Because this wasting gave no sign of fibre destruction Tower considered it an atrophy of disuse due to deficient movement and to deficient tonic innervation. Lippett also found (contralateral to an area 4 ablation) the brachioradialis more affected than any other muscle (loss one-third of its weight) except the coracobrachialis profundus (one-half). Although the rectus femoris had lost more weight than any of the remaining three divisions of the quadriceps femoris, its weight loss (one-seventh) was similar to that suffered by the sartorius and the adductors of the thigh, and less than the gastrocnemius (loss one-sixth of its weight) or the flexor carpi ulnaris (one-fourth). Wasting, therefore, appears to follow upon lesions of the pyramidal tract regardless of the level of its destruction, but shortening succeeds lesion of extrapyramidal systems from the frontal lobe when combined with that of the pyramidal unit.

Does the differential distribution of paralysis and of phenomena of release produced by injury to the projection systems from the area frontalis agranularis follow a primate pattern? If so, then the pattern in the monkey should be similar to that in man, and this patterned defect in the adult monkey should resemble that observed in the normal immature animal.

5. SPASTIC PARALYSIS IN MAN, COMPARED WITH THE RESULTS OF LESIONS OF THE MACAQUE'S 'MOTOR' CORTEX

Spastic paralysis in man, as the term implies, presents two diverse elements, spasticity and paralysis. The spasticity (Walshe, 1929) includes three phenomena which resemble somewhat in their quality although not in their distribution, the phenomena of release in decerebrate rigidity. These three phenomena are muscular hypertonus, clonus, and brisk tendon reflexes. To these phenomena must be added associated movements, atrophy of skeletal muscle and contracture. Paralysis of skeletal muscle, produced by lesion of cortici-fugal fibres from the precentral gyrus, must be separated from hypertonus within the muscle under consideration and from the contracture of the antagonists of that particular muscle. For movements which depend upon the innervation of muscle by extrapyramidal projection systems are more frequently than not handicapped by the abnormal distribution of tone. It is the distribution of hypertonia which has in part set the pattern of paralysis in man subsequent to lesions involving the pyramidal projection unit.

(*a*) *Extrapyramidal movements*

In spastic paralysis maximal paralysis is also differential in its distribution, affecting in the upper extremity the abductors, elevators and protractors of the upper arm, the extensors of elbow and fingers, the supinator of the forearm and the opponens of the thumb. In the lower extremity maximal paralysis was assigned to the flexors of the proximal joints, to the dorsiflexors of the foot and toes (Walshe, 1929), and to the abductors and external rotators of the thigh (Foerster, 1936*a*).

Moreover, it is possible that the upper arm cannot be elevated, abducted or protracted because of the increased tone in the retractors and adductors; that the forearm cannot be supinated because of the hypertonia in the pronator teres and the flexor carpi ulnaris. After combination of partial resection of nerves and lengthening or transplantation of tendons Foerster's (1936) patients were able with great difficulty to elevate, protract, abduct the upper arm, extend the elbow and supinate the forearm. And although all movement was that of either flexor or extensor synergies in such patients, nevertheless they were able to lift a glass of water to their lips and drink. Foerster's synergies were beautifully illustrated more than 30 years earlier by Marinesco's drawings (1903) of the use of the hand and arm contralateral to a year-old ablation of the precentral gyrus. Asked to flex the elbow, this patient flexed the head, retracted the upper arm, flexed the elbow, the wrist and the fingers with the thumb in great adduction. Requested to pick up a pencil, the paretic hand approached the object with the fingers equally flexed and abducted, the wrist flexed, and the forearm pronated. When the fingers closed upon the object the flexion of the wrist and of the fingers increased. The pencil was held by adduction against a flexed index finger, the forearm in a position midway between pronation and supination, that is, the human used his fingers and forearm exactly in the manner in which the monkey held small pieces of food in the hand contralateral to the area 4 ablation.

Similar use of the muscles of the arm characterize the young of both man and monkey. The early reaching and grasping movements are alternate extensor and flexor synergies. Small objects are first held by simultaneous flexion of all digits. During the second month of a monkey's life (Hines, 1942), the approach to a desired object changes from flexion

and abduction of the fingers to extension and adduction, a signal of active pyramidal participation in the reaching act. Not until the fifth month is the thumb opposed to a single finger and later still, independent movements of the forearm and of the wrist appear.

The hypertonus of the adductors of the thigh makes abduction difficult, for Foerster's paraplegic patient was able to abduct the legs after the dorsal roots of L_2, L_3, L_5 and S_2 were cut. Again the foot can be dorsiflexed if the Achilles tendon be lengthened, and the knee flexed if a partial resection of the nerve supply to the quadriceps femoris be done. But the paralysis of the retractors of the thigh remains. In the monkey the muscles which show maximal paralysis are the retractors and adductors of the thigh, flexors of the knee, dorsiflexors and evertors of the foot, flexors and abductors of the toes, whether the interruption of the cortico-spinal system be achieved at the cortical or subcortical level. The difference in distribution of maximal paralysis is even less than appears, for although the flexors of the monkey's toes are more paralysed than the extensors, nevertheless he is unable to dorsoflex these digits, a picture of paralysis of digital muscles very similar to that given for man subsequent to ablation of a part of the leg area (Walshe, 1935).

(*b*) *Atrophy*

This was also differential in its distribution within the muscles of both man and monkey. Subsequent to lesion of the precentral gyrus, Marinesco (1898, 1903) listed the following muscles as atrophic: deltoid, pectoralis major, flexors and extensors of the arm (extensors more than flexors), supinator, interossei, lumbricales, extensors of the fingers, adductor of the thumb, retractors of the thigh and flexors of the knee. Lippett's analysis of loss of weight in muscles opposite an area 4 ablation suggests that this list for man may be incomplete, for it will be remembered that maximal wasting affected the brachioradialis, flexor carpi ulnaris, the adductors of the thigh and the plantar flexors of the ankle. In bilateral ablations of area 4, atrophy, as determined by muscle palpation, encroached seriously upon axial musculature, affecting in particular the extensors of the trunk, the vertebral adductors of the scapula, and the deltoid. The addition of area 6 to the bilateral ablation of 4 increased the severity of wasting in the muscles of the extremities and of the trunk, and the adductors of the first digits.

(*c*) *Tone*

Bilateral division of the pyramids in the monkey was described as producing a hypotonia in which all skeletal muscle below the lesion shares, but shares unequally. Shortly after the lesion was made the decrement in tone was great in the muscles of the axis and in those which attach the extremities to their respective girdles; this decrement in tone was greater in particular in the extensors of the arm, the abductors of the fingers, extensors of the foot, flexors and adductors of the toes than in their respective antagonists. Eventually tone increased especially in the extensors of the back and the adductors of the legs. Apart from this increment of tone all other skeletal muscle below the lesion in the bilateral pyramidal animal took a permanent loss.

Strictly speaking, the condition of spastic paralysis in man is not comparable with the results of cortical removal of area 4 in the monkey. Except in Marinesco's two patients and Bergmark's (1909) study of cortical and capsular lesions the cases of spastic paralysis which have furnished the data for the distribution of tonic increment in skeletal muscle

have been those caused by capsular lesions. The instances of surgical removal of area 4 in man have not been studied with this purpose in mind (cf. Sachs, 1935; Walshe, 1935; Foerster, 1936*a*). Consequently, the existence of only minor differences in distribution of hypertonus found in spastic paralysis in man and in that in the monkey caused by ablation of area 4 is all the more remarkable. Tonic increment was described in the adductors of the upper arm and in the flexors of the toes of man, but not in the monkey; on the other hand, increase of tone was found in the abductors of the upper arm in the monkey, but not in man. Both primates share a remarkable increase in tone in the flexor carpi ulnaris, not present in either in the flexor carpi radialis.

In the monkey the tonic increment caused by unilateral ablation of area 4 was increased in its severity but not in its distribution by the additional removal of one area 6. The addition of the second area 4 or of second areas 4 and 6 produced a tonic increment in the muscles already hypertonic on the side of the new lesion and in the homologous muscles opposite the new lesion, and in addition selected for tonic increment part of the axial musculature, namely, flexors of the pelvis, the abdominal muscles, the latissimus dorsi, the teres major, and the muscles of the tail.

In the infant monkey (Hines, 1942) the development of a normal distribution of tone in its skeletal muscle takes place through a series of tonic increments and decrements. With minor exceptions the distribution of increased and of decreased tone in the infant has a striking resemblance to the distribution of increased or decreased tone in the adult which has suffered ablation either of the whole area frontalis agranularis or of its posterior division, area 4. During early postnatal development a definite increase in tone was distributed in the extensors of the head, the flexors of the trunk, the protractors of the shoulder girdle, the retractors and abductors of the upper arm, the flexors of the elbow, wrist, and fingers, m. brachioradialis and m. flexor carpi ulnaris of the upper extremity, and in the abductors, external rotators and protractors of the thigh, the flexors of the knee, ankle, and toes, and in m. tibialis anterior of the lower extremity. This hypertonia decreased and disappeared from these muscles in the following order: (1) the flexors of the digits, (2) from the proximal muscles of the extremities, (3) the muscles which move the 2nd joints, and (4) the muscles which move the 3rd joints. Concomitant with this decrease in tone an increase in tone occurred in the extensors of the trunk and in the vertebral adductors of the scapula.

Indeed, the distribution of tone in somatic musculature of the infant macaque passed through three periods. The tonic innervation of the flexors and related muscle groups was allocated to the immaturity of cortical areas 6 and 4; that of spasticity and initiation of spontaneous movements proximally, to partial development of the pyramidal tract and incomplete development of the anterior border of area 4; and that of maturation of fixation and a dominance of discrete movements of distal muscles to the maturation of the pyramidal tract as well as those extrapyramidal projection systems which originate in part at least from the area frontalis agranularis. Consequently, differential lesions of this region produced an approach to some stage of the infantile condition.

(*d*) *Contracture*

Hypertonic muscles become contractured or shortened in the spastic paralysis both of man and of monkeys. Both primates share two types of contracture of such muscles: (1) a shortening in which the muscle has lost a great part of its ability to contract because

connective tissue has replaced muscle fibres, and (2) one in which the hypertonic muscle has not lost the major part of its contractile power. On the other hand, a third type of contracture was found in the monkey (flexors of knee, area 4 or areas 4 and 6), one in which the muscle does not resist passive stretching, is itself hypotonic, has lost a great degree of its contractile power, and yet locks at a length less than the greatest distance between its origin and its insertion. This type of shortening would occur in man if the leg were not kept extended at the knee. If the hemiplegic man is to walk again contracture of the hamstrings must be prevented at all lengths less than full extension of the knee.

Besides the similarity in distribution of contracture in hypertonic muscle groups in these two primates, there is another within single muscles. The contracture of m. brachioradialis and m. flexor carpi ulnaris is strikingly alike in man and in monkey. The degree of fixed pronation is less in the monkey than in man. Moreover, in Foerster's experience (1936) contracture of m. flexor carpi ulnaris prevented complete passive supination, a condition also true for the monkey. Further, m. tibialis anterior although hypertonic in the monkey is not shortened, and similarly in man this muscle is rarely found in contracture.

V. CONCLUSIONS

The frontal lobe of the macaque was shown to contain the neural mechanisms which initiate progression, maintain posture, and select the skeletal muscle or muscles to be innervated. Whether movement would remain normal if the frontal lobes of this primate were allowed undisputed control of subcortical motor centres as it did in the cat and rat when the frontal lobes alone were spared (cf. Bard & Brooks, 1934) is unknown.

Certainly in this primate paralysis resulted from injury to the pyramidal tract. The distribution of severity of motor impairment was similar no matter where the lesion of that system was placed. Conversely, removal of extrapyramidal systems of the frontal lobe without injury to the pyramidal unit did not produce paralysis, but combinations of both systems increased the motor impairment characteristic of lesion of the latter.

On the other hand, the collateral phenomena accompanying the destruction of the pyramidal unit at the cortical level shared only two moieties with those produced by surgical division of the pyramids, atrophy and associated movements. The distinctive collateral phenomena of cortical ablation of this system are hypertonus, brisk irradiating reflexes, clonus, and contracture; that of pyramidal division, hypotonus.

Atrophy of skeletal muscle followed pyramidal section. Accompanied by contracture, atrophy followed removals of area 4, and became severe particularly in the musculature of the terminal appendages subsequent to additional ablation of area 6. Topical lesions of the posterior division of area 4 in the mature macaque caused atrophy within the contralateral paretic extremity. In general, greater degrees of paralysis were accompanied by greater degrees of atrophy and supported Tower's (cf. also Tower, 1939) correlation of the atrophy in pyramidal lesion with disuse.

Associated movements were shown to be mass movements of extrapyramidal innervation which became evident by removal of pyramidal action. Removal either of small parts of the pyramidal unit or of extrapyramidal frontal lobe fields did not produce associated movements. The variety of these movements characteristic of pyramidal section was decreased by inclusion of extrapyramidal projection units in the ablation.

The tonic increment so produced was probably the associated factor; for addition of ablation of the parietal lobe to ablation of area 4 resulted in tonic decrement and increase in the number of associated movements. On the other hand, these mass movements required the presence of some part of frontal lobe: one area 4 or one prefrontal area would suffice.

Three types of resistance to passive stretching of a muscle were described: (1) hypertonus so great that it constituted rigidity, (2) the 'clasp-knife' type of resistance, confined to the middle 30–40 degrees of the arc of stretching, and (3) a resistance operating evenly throughout the total stretching arc. The first, produced by bilateral frontal lobectomy, was distributed alike to all muscles of the extremities and confined in the trunk to its ventral muscles. The 'clasp-knife' type of hypertonus was caused by ablation of area 4 (whole or anterior division) or by removal of area 6 and the posterior division of 4. The distribution of the hypertonus resulting from the former resembled that characteristic of man's spastic paralysis; that of the latter was the reverse of the former at the contralateral knee and elbow. The third type was due to removal of area 6 and the anterior border of area 4 and was distributed alike between the flexors and extensors of the contralateral knee and elbow. Each of these operations in the monkey produced at the extreme distal and proximal joints (i.e. excluding the knee and elbow) a hypertonus distributed similarly to that of spastic paralysis in man. The differences recorded at the extremes of the extremities was quantitative, not qualitative—a condition which might be complicated by contracture, particularly of muscles of the wrist and ankle.

Contracture of skeletal muscle produced by suprasegmental lesions cannot be reduced (with the data at hand) to a common denominator. The early savants of clinical neurology allocated contracture in central nervous system lesions to abnormal excitation of ventral horn cells due to irritation set up by degeneration of the pyramidal tract (cf. Marinesco, 1898), or to over reaction of spinal centres (cf. Mann, 1897): the muscles least paralysed became contractured. Gowers (1893) suggested that the centres which overact were those on which normal muscle tone depended and that the contracture or shortening was due to an excessive degree of this tone. Because Tower (1940) found no trace of contracture accompanying the muscular atrophy of pyramidal lesion she concluded that contracture of suprasegmental lesions proceeded from overactivity. Overactivity therefore was assigned by these two writers to an excessive degree of tone. Moreover, in the monkey not all hypertonic muscles were contractured and not all contractured muscles were hypertonic.

The degree and distribution of contracture in the monkey depended upon the cortical loss of extrapyramidal systems in the presence of severe pyramidal defect, and therefore related in part to the degree and distribution of hypertonus. However, the severe contracture which followed shortly upon the completion of bilateral ablation of areas 4 and 6 began to decrease with return of power of progression. But this diminution in degree of contracture ceased before improvement in movement impairment stopped. Does the fact that the degree of paralysis characteristic of the primate does not accompany the hypertonic states produced in laboratory mammalian quadrupeds by removal of the motor cortex (Woolsey, 1933), or of the frontal area and the anterior lobe of the cerebellum (Snider & Woolsey, 1941) or of the whole cortex (Bard, 1942), account for the absence of contracture in these forms? Or is another factor either present or absent in the laboratory quadruped which spares it the contracture (cf. also Munk, 1895) which

these cortical losses produce in the monkey? Paralysis in the latter might be considered to play its part by a non-interruption of the hypertonic state and yet the clinicians have concluded that the least paralysed muscles became contractured. If hypertonus be synonymous with overactivity, some collateral factor or factors as yet unknown must select certain hypertonic muscles for contracture and leave certain others free. This unknown collateral factor might be the one on which contracture of hypotonic muscle depended.

A limited correlation of architectonics and cortici-fugal systems with the results of electrical stimulation and of cortical ablation is possible. The purposive use of skeletal muscle depended upon the intactness of the area frontalis agranularis, the purposive use of isolated muscles upon area 4. Electrical stimulation of these areas yielded positive results as movements; cortical ablation, negative results, the loss of these movements. Discrete movements were absent to electrical stimulation, and in the animals use of somatic musculature after division of the pyramids in spite of an intact cortical surface. The function of the remaining isogranular cortex classed as motor cannot be decided at present.

The whole area frontalis agranularis shares cortici-fugal terminations in the subthalamic tegmentum, the substantia nigra, the formatio reticularis and pontine nuclei. Electrical stimulation of area 6 as well as its anterior and posterior borders elicited flexor synergies with grasping, increase in flexor tone and relaxation of the grasp: removal of area 6 alone caused the appearance of the grasp reflex. Electrical stimulation of the anterior border of 4 as well as the posterior border of area 6 evoked extensor synergies frequently bilateral, diagonally organized movements, an increase in extensor tone, and inhibition of standing tone: removal of the anterior border of 4 (4*s*) produced brisk irradiating tendon reflexes, hypertonus of the 'clasp-knife' type differentially distributed, clonus and a minimal residual paralysis. In a loose way the results of separate removal of these two areas were the reciprocals of those of electrical stimulation. The correct identification of the cortici-fugal systems involved in each case is impossible.

However, both the nucleus ruber and the substantia nigra have undergone marked phylogenetic development especially in primates, transforming them from simple groups of tegmental motor nuclei to centres of integration for motor activity. Areas 6, 4*s*, and posterior 4 share terminations within the substantia nigra, suggesting that the non-adversive extrapyramidal movements may have been evoked through this nucleus. Had an occipito-rubral system from areas 18 and/or 19 been described the cortical surfaces from which orienting movements of adversion were obtained would have shared a common termination in that nucleus. All cortical areas from which movements of eyes and ears were obtained sent fibres into the bulb; but for frontal orientation choice lay between a cortico-bulbar tract and one terminating in the formatio reticularis of the medulla oblongata.

The inhibitory action obtained from the cortical surface of areas 4*s*, 6, and 8 (pyramids severed) was non-topical and bilateral. These regions share appreciable projection systems to the substantia nigra and the formatio reticularis. But from 4*s* the cortico-tegmental fibres ending in the midbrain formed a large bundle, while those from posterior 4, areas 6 and 8, consisted of a few scattered fibres, suggesting that the former might carry the inhibition of standing tone which was exaggerated subsequent to 4*s* ablation. That the brisk irradiating reflexes, clonus, and hypertonus produced by removal of this

area were not dependent upon a single fibre unit was suggested by the differential regression of each of these elements during the first three months of the infant monkey's life. The allocation to a particular cortici-fugal unit of the inhibition of flexor tone and the grasp obtained from area 6 was complicated by the finding that cortici-fugal fibres from this area and the posterior part of 4 have similar terminations. This fact may account for the apparent summation effect produced by simultaneous ablations of these areas. All the cortical areas which yielded quieting projected upon the lateral nucleus of the thalamus.

The classification of muscles used in the present article was based upon the position of their innervating nuclei within the spinal cord (cf. Bok, 1928; Bikeles, 1905). The muscles derived from the dorsal sheet of mesoderm, the extensors and abductors, were innervated by nuclei within the ventral half of the lateral division of the ventral horn and those derived from the ventral sheet of mesoderm, the flexors and adductors, were innervated by nuclei within the dorsal half of the lateral division of the ventral horn. Using this classification the hypertonus of spastic paralysis of both man and monkey was distributed in the flexors and adductors of both extremities and in the retractors of the arm and the protractors of the leg, with a few significant exceptions. In both primates the hypertonus was found in m. quadriceps femoris rather than the flexors of the knee: in the monkey m. brachioradialis and m. tibialis anterior were added to this list and the adductors of the upper arm and the flexors of the toes subtracted.

The variation in distribution of hypertonus produced by circumscribed cortical lesions of the monkey's frontal lobe was confined to the muscles of the second joint. Here only the distribution of hypertonus characteristic of the lesion changed with posture. The distribution found in the sitting posture changed when the extremity was used for support: occurring in the flexors the hypertonus shifted to the extensors, occurring in the extensors, or in flexors and extensors alike, it increased in the extensors. Brain (1927) described a similar change in the arm of the human hemiplegic, for when required to support the weight of the upper part of the trunk the hypertonus shifted from the flexors to the extensors of the elbow.

Man as a primate is more closely related to the macaque than he is to either the cat or the dog. When the infant monkey learns to walk (cf. Hines, 1942, fig. 29) he supports the body by extremities in flexion and abduction, the arm in retraction and the leg in protraction, tail and trunk in ventriflexion and head extended. With each protraction of the leg the tail abducts and the trunk flexes laterally. Man's earliest progression resembles the monkey with one important difference, the extremities are adducted. The bilateral 4 and 6 preparation approaches but never realizes the infantile mode of progression, for the legs (not the arms) are greatly adducted. The monkey's earliest infantile posture resembles that of a reptilian quadruped. Indeed, the distribution of hypertonus produced by loss of the 'motor' cortex is distributed in the muscles which control early progression in these two primates, with the exception of m. quadriceps femoris in both, the tibialis anterior and the adductors of the thigh in the monkey. Viewed in this manner no complete 'resetting' of the spinal cord nuclei (Walshe, 1929) is necessary to explain the distribution of hypertonus in man, as would be required if man be compared to a cat or a dog and antigravity muscles regardless of origin be classified as extensors. Considered in this way the 'resetting' of spinal nuclei in man is related to his upright posture and affects mainly the muscles of the lower extremity. This concept of 'resetting', using

the reptilian quadruped as the prototype, is limited mainly to the muscles of the knee and, as so limited, is strengthened by the finding that the type and distribution of hypertonus caused by cortical lesions in the monkey vary at the second joint only. And as a corollary we note that the greater degree of the paralysis in proximal muscles (man or monkey) affects the retractors and adductors of the thigh and the flexors of the knee, muscles which participate in the phasic element of this postural change and seem to have become dependent upon the cortex for their innervation. In this connexion it is interesting to note that Foerster (1936) found mass representation in the human precentral gyrus to favour the flexors of both extremities with two exceptions only, the dorsiflexors (i.e. extensors) of the ankle and the extensors of the knee.

Electrical stimulation of the cortical surface of the monkey after surgical division of the pyramids so far has never elicited (cf. Tower & Hines, 1942) all the patterns of movement observed in the unanaesthetized chronic preparation, as it did in the cat. Furthermore, all of the patterns of movement elicited by stimulation of the cortical surface in the bilateral pyramidal cat was also observed in the decorticate cat (cf. Tower, 1936, p. 441). No such similarity exists in the monkey. The decorticate monkey is helpless (the writer was allowed to examine the beautiful preparation kept alive by Dr C. N. Woolsey for 161 days). This discrepancy between the extrapyramidal organization observed in the unanaesthetized bilateral pyramidal monkey and the results of direct stimulation of cortici-fugal systems is subject to two interpretations. Either the conditions under which these stimulations are performed preclude duplication in the monkey, but not in the cat, or extrapyramidal activity is organized at the cortical level in the monkey rather than at the subcortical level as it is in the cat. A migration of the centre for integration of extrapyramidal movement could account for this difference between the patterns of motor performance observed in the unanaesthetized bilateral pyramidal monkey and the movements elicited by electrical stimulation of its cortical surface.

In this primate the cortex cerebri posterior to the central fissure has a remarkable and intricate localization of sensory function (cf. Walker, 1938; Woolsey, Marshall & Bard, 1942; Le Gros Clark, 1941; Talbot & Marshall, 1941) with scant provision for motor activity. Anterior to this fissure the frontal lobe has virtually cornered the control of movement. In particular, the anterior border of area 4, area 6, area 8*s*, and possibly a part of 9 have assumed the integration of the least stereotyped extrapyramidal action and the control of tonic inhibition unassociated with that of the pyramidal unit itself.

Therefore the control of movement exercised by the frontal lobe in the monkey is the resultant of interplay of two types of cortico-fugal units, one specific, the pyramidal, and one general, the extrapyramidal. The contribution made by the former requires that made by the latter, for the incomparable contribution of the pyramidal system demands some control of tone. Working together, these systems allow man or monkey to innervate groups of muscles or to pick out a single muscle for action with appropriate degrees of tone or of movement in co-operating muscles. Although grading the power of contraction may be the pyramid's contribution to innervation of more proximal muscles the 'fusillade' innervation of limb musculature, so important in the initiation of movements of the co-operating extremity in the performance of skilled movements by the active extremity, is probably extrapyramidal in origin. Were it surgically possible to divide extrapyramidal pathways, leaving the pyramidal unit and the cortex intact, this review

would have to be rewritten, for then we might know how to assign properly to each unit its own contribution to tone and to movement.

VI. SUMMARY

Only the area frontalis agranularis of the agranular isocortex allocated by Economo & Koskinas to the cytoarchitectural motor type of cortex (man and macaque) has yielded contraction of skeletal muscle by stimulation with electric currents. Regions surrounding Flechsig's primary areas, characterized by large pyramids in layer III*c*, were electrically excitable in the macaque; but in man no movements so far have been obtained from areas 18 and 22. Areas which share no cytoarchitectural features of these electrically excitable regions have responded to stimulation with the electric current. With one exception it is impossible to allocate these results to particular cortici-fugal systems. Regions from which movements of eyes and ears were obtained shared either cortico-tectal or cortico-bulbar systems.

The frontal lobe of the monkey contains the neural mechanisms which maintain posture, start progression and choose the skeletal muscle to be innervated. The purposive use of that muscle in mass organization depends upon extrapyramidal systems; that in discrete organization upon the pyramidal system. Atrophy of skeletal muscle followed lesion of the pyramidal tract; hypertonia, contracture, brisk tendon reflexes, and clonus, followed ablation of extrapyramidal systems stemming from area 4; the 'grasp reflex' (reflex contraction of flexor muscles maintained by stretch) followed removal of extra-pyramidal systems from area 6.

Coincident with the remarkable mosaic type of localization of sensibilities within the three posterior lobes, control of movement, the initiation of contraction, fixation of more proximal muscles, distribution of tone, sequence of contraction with innervation of prime movers and of co-operating muscles have migrated as a whole to the frontal lobe of the primate, *Macaca mulatta*. Compared with the cat, in which extrapyramidal activity is distributed generously in three of the four lobes of the cortex cerebri, the frontal lobe of the monkey, like that of man, 'serves in the motor aspect of the mind'.

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POLYGENIC INHERITANCE AND NATURAL SELECTION

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CONTENTS

I. NATURAL SELECTION

Evolution is the occurrence of persistent changes in the hereditary constitution of a population of organisms. Two conditions must be fulfilled if such changes are to occur by natural selection.

The first is that the population shall be, or shall be capable of becoming, genetically heterogeneous; for if, except for non-heritable variation, all the individuals are alike, and are incapable of changing, their progeny must also be alike and evolutionary changes become impossible.

The second condition is that genetically unlike types leave different average numbers of mature progeny in the next generation. Otherwise, natural selection would be inoperative, and evolutionary changes would depend, of necessity, on new variation being directional and preponderantly adaptive. Genetical evidence (see Muller, 1939*) indicates that this is not the case, mutation being a process which, as Darlington (1937) has said, is chemically determinate but biologically at random. With new variation not

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always directly adaptive, selective breeding is the necessary condition for sorting out advantageous and disadvantageous variants.

Now if these two conditions are fulfilled natural selection must occur (Fisher, 1930*a*), but this does not imply that evolution proceeds by this process. Unless the variation subjected to selection is of an appropriate kind, and unless the selective breeding is itself suitable, the effects of natural selection may be trivial and incapable of supplying a mechanism of evolutionary change. Thus, for example, selection is known to favour heterozygotes at the expense of homozygotes in some cases (Fisher, 1939); but this can only lead to the maintenance of a special kind of polymorphism. Evolutionary change cannot be a consequence of this particular kind of selective breeding. Natural selection is a phenomenon separable from evolution and capable of being studied independently. It is on such study, attempted by Fisher (1930*a*), that the demonstration of evolution by natural selection depends.

Any investigation of the mechanism of natural selection or of evolution must ultimately be genetical, for it must involve the separation of hereditary from non-hereditary variation and the analysis of the former's changes in succeeding generations under the action of changing conditions. Genetics has already contributed materially to our understanding of these processes, notably in removing one of Darwin's greatest difficulties by the demonstration that inheritance is particulate and hence that the decay of heritable variability in a population is slow (Fisher, 1932).

During the last ten years more attention than ever before has been devoted to the genetical study of populations, and the consequent contribution to evolutionary theory has been great. The validity of the two fundamental theorems of natural selection has been fully verified. Evidence of genetical heterogeneity in populations will be reviewed later (§ II (2)) and that of the differing selective advantage of various genotypes has been given by Fisher (1939) and Ford (1940*b*). It has also been possible to go further and show that, so far, nothing has been discovered about evolutionary change which is in conflict with, or demands an extension of, the known genetical principles of variation and natural selection (Timoféeff-Ressovsky, 1940; Muller, 1940; Darlington, 1940). The application of genetics to the detailed analysis of evolutionary change has commenced to bear fruit. In order to see how fully the genetical principles of variation and natural selection can account for evolutionary changes, we must examine the nature of specific differences in relation to intra-specific variation, the way in which variability is maintained in populations and how responses to selection occur. Many of the questions which arise will be capable of more critical analysis as new facts become available in the future; but we can recognize now that the success of such analyses must depend on their being treated as special aspects of the general problem raised by natural selection.

II. NATURAL VARIATION

(1) *Specific differences*

Specific differences cannot commonly be subjected to genetical analysis, because individuals of different species will seldom hybridize, and, even when obtained, species hybrids are very often infertile. Thus information on the genetical nature of specific differences is somewhat scarce. Some evidence has, however, been obtained, and it is

clear that several types of difference are involved. The problem now is that of deciding which of these are characteristic of the distinction between species.

Polyploidy will be omitted from the discussion. Though in many plant genera this phenomenon has played a great evolutionary part (Darlington, 1937; Dobzhansky, 1941), polyploidy has been of very minor importance in animals, and in any case, results more in combining the features of two existing species, than in initiating entirely new forms. Structural changes, such as inversions, interchanges and duplications, will also be omitted, except for a few special cases mentioned later. Selection acts directly on the genic constitution of the organism, structural rearrangements being affected only in so far as they control or determine the behaviour of individual genes. Our main concern must clearly be that of finding out how new genotypic systems are brought into being. This will be approached using only the concepts of formal genetics, to which cytological theory is ancillary for our purpose.

Before proceeding to a consideration of existing data it is necessary to decide on the criterion by which the importance to specific distinction of various types of genetical difference are to be judged. If, as the theory of evolution by natural selection holds, species differences are to be found in embryo in racial and varietal differences, genetical variation of the kind found between species must also be of common occurrence within species; but it is clear that this variation within species, though of the same kind, must be of smaller magnitude or less extensive than the variation between species. Otherwise species differences would be genetically no more significant than intra-specific variation and the problem would not exist. The application of this criterion will be clearer when the evidence has been discussed.

First of all, differences distinguishing species may be oligogenic, i.e. they may be controlled by a small number of genes having effects large when compared with non-heritable fluctuation, and hence leading to sharp segregation. (The familiar genetical variants of the laboratory are of this kind.) Examples of oligogenic variation between species are not uncommon. Green (1935), for example, has shown that *Mus bactrianus* has the character white-bellied agouti, while many populations of *M. musculus* have the allelomorph giving common agouti. *Petunia integrifolia* has red flowers, differing from the white flowers of *P. axillaris* by two genes (Mather, unpublished). Similar flower-colour differences are also involved in *Streptocarpus* species (Lawrence, Scott-Moncrieff & Sturgess, 1939) and *Nemesia* species (Mather, unpublished). Other characters may show the same behaviour, as, for example, habit in crosses between *Phaseolus vulgaris* and *Ph. multiflorus* (Lamprecht, 1941). All these cases and others like them were found as a result of species hybridization, but a case in *Drosophila* is of interest as having been detected in a different way. Gottschewski & Tan (1938) have rendered likely the existence of a monogenic difference in eye colour between *D. melanogaster* and *D. pseudo-obscura* by transplantation experiments, the two species being incapable of crossing.

Oligogenic differences do not, however, satisfy our criterion, for species are known which need not differ in characters of this kind. Furthermore, differences of exactly the same kind as those distinguishing two species may be found within either or both of them. Indeed, differences of this kind, whose study has formed the main occupation of geneticists, are more common within than between species. There is no relation between closeness of relationship and number of oligogenic differences. Particular oligogenic variation seems, rather, to be an outcome of the possession of particular hereditary

material in the chromosomes, for similar variants are often found in related species, genera or even higher groups (see Haldane, 1927*; and Vavilov, 1922), where it has given rise to Vavilov's 'Law of Homologous Variation'.

A second type of heritable difference shown by species is dependent on the joint action of many genes, each having an effect small in relation to the total non-heritable fluctuation of the character in question. Such differences are termed polygenic, and polygenic characters do not show sharp segregation. They may exhibit any degree of expression between wide limits and hence have often been called quantitative characters. Size and shape are typically polygenic in most cases. Polygenes are inherited in exactly the same way as other genes, in that they are situated in the chromosomes (Warren, 1924; Mather, 1942*b*). They differ only in the type and magnitude of phenotypic effect produced.

The absence of simple sharp segregation for polygenic characters has made their exact genetical study difficult, but cases of polygenic specific differences are well known. Perhaps the most familiar is that described by Baur (1932) in *Antirrhinum*, but almost every account of species hybridization refers to complex segregations for some characters. Evidence has been given in detail by Harland (1936*) for *Gossypium*, Anderson (1939) and Smith (1937) for *Nicotiana*, Honing (1923, 1928) for *Canna*, Green (1935) for *Mus*, Iljin (1941) for *Canis* and others. Although data are absent in many cases, it seems to be generally agreed by all who have experience of species hybrids that they are characterized by polygenic segregation of size and shape characters (see especially East, 1935; Timoféeff-Ressovsky, 1940; and Muller, 1940).

Polygenic variations are known within species, as we shall see in the next section, the differences being smaller than those between species. It thus appears that polygenic differences fulfil the criterion and may be regarded as essential to specific distinction.

A third type of specific variation is described by Harland (1936) in *Gossypium*. Related species have mutant forms which can be shown to be dependent on mutation of homologous genes. Individuals of the two species often differ slightly when heterozygous for the mutant, so showing that their wild type, or commonly occurring, allelomorphs are not quite the same. It is of course possible that the differences are due to other genes very closely linked to the one in question, but, in any case, the differences between homozygotes are too small to add appreciably to the specific distinction, except, perhaps, as part of a polygenic combination.

A final type of interspecific difference must be mentioned. In some plant genera, notably *Epilobium* (Michaelis, 1937), *Streptocarpus* (Oehlkers, 1938) and *Oenothera* (Renner, 1936), species differ cytoplasmically as well as genetically. Cytoplasmic variation is not, however, a regular and characteristic feature of species distinction. It is apparently absent in most cases.

The genera *Oenothera* and *Triticum* (and possibly some others) require special mention, for they seem to constitute exceptions to the rule that specific differences are essentially polygenic. In each genus particular species appear to differ only by a single major heritable factor (Watkins, 1930; Ellerton, 1939; Renner, 1925). Special reasons for this behaviour are, however, known in *Oenothera*. Many of the species, like *Oe. Lamarckiana*, are permanent hybrids, whose chromosomes behave in such a way that recombination in the differential segments is suppressed. In consequence, apparently

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unifactorial segregation is inevitable, no matter how many genes are really involved (Darlington, 1931, 1939). The evidence of aberrant cytological behaviour in *Triticum* is less strong, but it seems likely that here too the differentiating genes are inherited as a complex. The anomalous nature of the species differences in these genera is probably more apparent than real, the general rule still holding good.

(2) *Variation within species*

All the types of heritable variation by which species may differ are also found within species. Oligogenic variation of two types is to be observed. The first kind, whose existence has long been recognized, gives polymorphism. This is widespread among butterflies (Ford, 1940*b*) and is established in some of the Coleoptera (Timoféeff-Ressovsky, 1940; Dobzhansky, 1933), in *Helix* (Diver, 1940), *Lebistes* (Winge, 1927), the grouse locusts, *Paratettix*, *Apotettix* and *Acrydium* (Nabours, 1929; Nabours, Larson & Hartwig, 1933), the grasshopper, *Chorthippus parallelus* (Sansome & La Cour, 1935), *Lotus* species (Dawson, 1941) and elsewhere (Timoféeff-Ressovsky, 1940; Ford, 1940*b*). Many other organisms, both plant and animal, most probably fall into this class, though conclusive genetical evidence is lacking. In some cases the same polymorphic variant that is found within a species also serves to distinguish two related species. It is not, however, clear whether the polymorphic gene is an essential part of the species difference in such cases (Timoféeff-Ressovsky, 1940). It may be noted that certain examples of Huxley's (1939) 'clines' and the wild inversions in *Drosophila pseudo-obscura* (Dobzhansky & Sturtevant, 1938) are special examples of polymorphic variation. Polymorphism depends on a curiously balanced set of selective advantages (Fisher, 1927; Ford, 1940*b*), and there is reason to believe that this balance, and hence polymorphism, may depend for its maintenance on polygenic differences (see § V (1)).

The existence of the second type of oligogenic variation has been established more recently. Mutant genes of the type found and used in laboratory studies have been detected in wild material of several species of *Drosophila* (reviewed by Dobzhansky, 1939*), *Dermestes* (Philip, 1938), *Gammarus* (Spooner, 1932), rats (Lloyd, 1912), *Peromyscus* (Sumner, 1932), *Trifolium* (Williams, 1935) and grasses (Jenkin, 1930), in fact wherever a serious search has been made. The variants, which include lethals, sub-lethals and various visible colour and structural aberrants, are rarely to be observed homozygous in wild individuals, though some examples have been seen. A very large proportion of wild organisms have, however, been found by adequate tests, to be heterozygous for the mutants. Many of these genes reduce the viability of the carrier, and in any case, as we have seen, there is no evidence of specific difference being dependent on this type of variation. Hence we cannot regard this type of oligogenic variation as having any adaptive or evolutionary significance in the general case.

Naturally occurring polygenic variation within species has been less analysed than the oligogenic type. It is, however, clear that variation of this kind is widespread. The biometrical studies made in man by Galton, Pearson and their associates have disclosed a wealth of variation suggestive of the polygenic interpretation (cf. Fisher, 1918), and nearly all important characters such as yield, quality and disease resistance in domestic animals and plants are of this type (Emerson & East, 1913; Rasmusson, 1933; Hammond, 1940).

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In *Peromyscus* the characters distinguishing races appear to be polygenic (Summer, 1932). Homologous oligogenic variants were, on the other hand, found in many races, again showing how different in evolutionary importance the two types of variation are. The races of *Drosophila pseudo-obscura* also differ polygenically, even in respect of their interfertility (Dobzhansky, 1936). Polygenic morphological differences observed between the races of this species are greater than those found between strains of the same race (Mather & Dobzhansky, 1939) but less than those between this and related species such as *D. miranda*. Local groups of *D. melanogaster* are distinguished by their numbers of sternopleural chaetae (Dubinin *et al.* 1934), which Wigan (1941) has shown to be subject to polygenic control, a great deal of variation being present in wild flies.

In many cases, though the genetical data are inconclusive, wild individuals or their offspring have been found to differ in those characters, such as size, shape and growth habit, which are commonly subject to polygenic variation. There is little doubt that intra- like interspecific variation is polygenic, though the degree of variation is not so great.

A case of allelic variation, such as Harland described between different species of cotton, has been found within *D. melanogaster*. Two strains, one American and the other Russian, had, at the white locus, wild-type allelomorphs distinguishable from one another by their mutation and dominance characteristics (Timoféeff-Ressovsky, 1932; Muller, 1935). This is, however, a unique example outside cotton.

Cytoplasmically inherited variants are known within a number of species (Sirks, 1938), but they are not so common as the more familiar nuclear kind. Racial differences may be cytoplasmic as in the case of *Vicia faba major* and *minor* (Sirks, 1931), but this is not characteristic of racial differentiation (cf. *Drosophila pseudo-obscura*), and, as in the case of species differences, cytoplasmic distinctions between races seem to be exceptional rather than regular.

Finally, there exists within species a kind of variation which cannot develop directly into interspecific difference, viz. polymorphism affecting the breeding system, or system of mating, within the species. Related species may differ in their breeding systems, but the variation on which the control of breeding rests is always, of necessity, intraspecific. The most familiar kind, indeed the most familiar kind of all genetic variation, is sex separation, but others, e.g. incompatibility of the *Nicotiana* type (East, 1929) and heterostyly (see Darwin, 1877), are quite common. In the cases of sex separation and heterostyly the differences between male and female or between pin and thrum, are morphologically obvious, though certain other mechanisms also play a part as we shall see later (§§ VI (1) and (2)). Incompatibility, or, as it is often called, self-sterility, depends on a physiological reaction between pollen and style. It involves no morphological differentiation and so was not understood until relatively recently in *Nicotiana*. Since that time it has been found to be of widespread occurrence in plants.

Other mechanisms controlling the rates of outcrossing and inbreeding also exist (see Kerner & Oliver, 1894–5) and probably involve some genetical variation, though little accurate knowledge of their genetics is available. Lewis (1941) has discussed the genetical possibilities of gynodioecy and concluded that it must depend on cytoplasmic differentiation if it is successfully to function as a means of controlling the breeding system.

This type of polymorphism is of special interest because both its adaptive value and genetical stabilization are more obvious than those of the other types of polymorphism

mentioned earlier. All the various systems play the part of controlling outbreeding or inbreeding, and hence of affecting the average heterozygosity of the organism. The precise system operating in any species is determined by the existing morphological and physiological peculiarities (Mather, 1940). The question of how breeding mechanisms affect heritable variability, of how they are adapted to fresh circumstances and of the effects of these adaptive changes, will be discussed after the properties of polygenic inheritance have been considered.

III. POLYGENIC INHERITANCE

(1) *Phenotype and genotype*

Evolutionary and adaptive changes are dependent on polygenic characters, and so our attention must be directed towards polygenic variation and its behaviour under the action of natural selection. Polygenic characters are controlled by many genes having effects small in comparison with non-heritable fluctuations. In consequence, polygenic inheritance is marked by certain peculiar features which distinguish it from oligogenic behaviour and which throw a fresh light on the interrelations of variation and selection. Polygenetics represents a new level of integration by means of which a better understanding of natural selection and its action may be achieved.

The quantitative difference in number of operative genes between polygenic and oligogenic variation gives a qualitative difference in behaviour. Laboratory genetics has been almost solely concerned with oligogenic variation, and so has proved disappointing to the evolutionist. Both types of gene are, however, inherited in the same way and so the success of polygenic analysis depends on the utilization of the principles elucidated in laboratory studies.

Before proceeding to a discussion of polygenic inheritance, one point must be made clear. Any given character may be subject to both polygenic and oligogenic variation. Thus a *Drosophila melanogaster* may be wild type and have some 18 or 20 chaetae on the ventral surface of each abdominal segment, but it may, on the other hand, show the effects of the mutant gene 'scute', in which case the number of chaetae is very much smaller. The flies of each kind are sharply distinct, for, though the chaeta number is variable, the two classes, wild type and scute, do not overlap. This is characteristic of oligogenic variation. But the precise number of chaetae on a wild-type fly is subject to the control of many genes each of small effect, as well as being influenced by environmental conditions. The continuous phenotypic variation produced in this way is characteristically polygenic. The number of polygenes is not known, but small-scale experiments have shown them to lie in all the major chromosomes (Mather, 1942*b*). Wild flies commonly show polygenic variation of this character, but oligogenic variation is very rare.

When a single gene is involved, zygotes must fall into three genotypic classes, the two homozygotes and the heterozygote. With incomplete dominance this means that the maximum number of three phenotypes will be found. With dominance the number of phenotypes is reduced to two, one of which will include both the homozygote **AA** and the heterozygote **Aa**. In such cases one phenotype, usually that associated with the dominant allelomorph, will be selectively advantageous when compared with the other. The disadvantageous allelomorph must tend to become less and less frequent until it is only maintained by mutation as a rarity in the population. No finer adjustment is possible.

Where, however, a number of polygenes is involved, the situation is very different, for many phenotypes are possible, a large proportion of which will be produced by a number of different genotypes. With only three polygenes of equal effect, the genotypes **AABBcc**, **AAbbCC** and **aaBBCC** will, for example, give the same phenotype. This phenotype would also characterize the genotypes **AaBBcc**, **AABbcc**, **AaBbcc**, etc., if dominance were the rule, or **AABbCc**, **AaBBCc**, and **AaBbCC** in the absence of dominance. With more genes the possibilities are increased.

Two important consequences then follow. First, neither allelomorph of a polygene will have an unconditional advantage over the other, for each may form part of a distinct genotype giving the same phenotype. Thus, in the example considered above, **AAbbCC** and **aaBBCC** gave the same phenotype as each other. So would **AAbbcc** and **aaBBcc**. No matter which of these two phenotypes is the more advantageous, **A** will sometimes be favoured at the expense of **a**, while the reverse will hold in other cases. The same is true of **B**, **b**. The selective properties of one polygene will be conditioned by the other polygenes which are heterogeneous, i.e. exist as at least two allelomorphs, in the population in question. The second consequence is that a very fine adjustment of phenotype to environment becomes possible, for the chance of finding a phenotype closely adapted to the prevailing conditions increases with the number of phenotypes which can occur. When circumstances change, a different phenotype will show maximum adaptation; but whatever the conditions, close adjustment is possible. Polygenic variation gives great adaptability, the nearly continuous variation permitting more regular and more accurate adaptation.

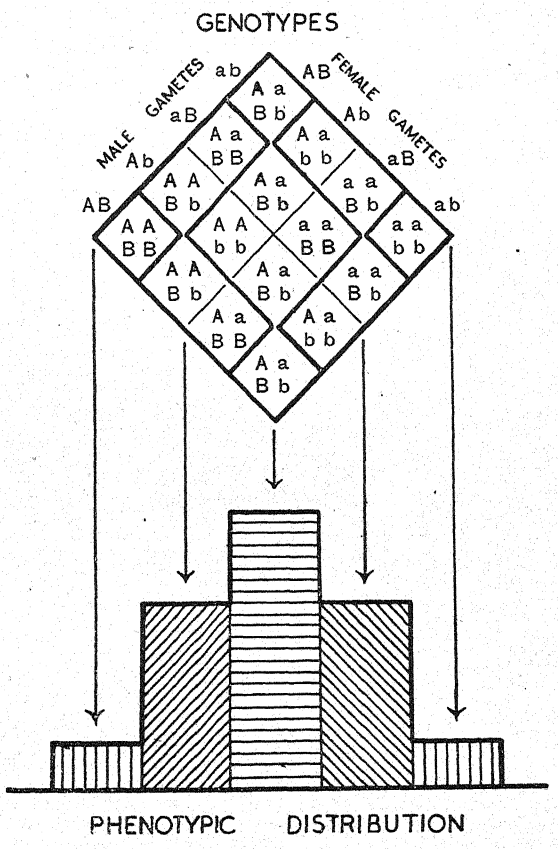

Fig. 1. Genotype and phenotype. The phenotypic frequency distribution of a character controlled by two genes of equal and independent effect, without dominance. The phenotypic expression is proportional to the number of capital letters in the genotype (see text).

The different phenotypes will not be equally frequent in a population for two reasons. First, the numbers of genotypes which give rise to particular phenotypes are not constant, and secondly, the various genotypes have different frequencies of occurrence. This can be well seen from the simple example of a character controlled by two incompletely dominant independent genes, **A**, **a** and **B**, **b**, the two allelomorphs at each locus being equally frequent (Fig. 1). The allelomorphs designated by small letters are assumed to add nothing to the expression of the character, while each allelomorph

designated by a capital letter adds 1 unit. Fig. 1 shows the gametes and zygotes which will be produced as a result of random mating in such a case. The zygotes fall into nine genotypes, four of which each occur in 1/16 of cases, four more in 2/16 of cases and one in 4/16 of cases.

There are, however, only five phenotypes, the two extreme and least frequent ones being produced by only one genotype each, viz. **aabb** and **AABB**. The central and most frequent phenotype is given by three genotypes, **AaBb**, **AAbb** and **aaBB**, the first of which is itself the commonest genotype. The remaining two phenotypes are each produced by two genotypes of intermediate frequency, **Aabb** and **aaBb** in one case and **AABb** and **AaBB** in the other.

This type of phenotypic frequency distribution is characteristic of polygenic inheritance. As the number of genes involved increases, more phenotypes are possible, and the distribution becomes more nearly continuous. With many genes, and with non-heritable influences playing a part, the distribution must often approximate to the normal as observed, for example, in human stature.

(2) *Dominance and interaction of polygenes*

The exact shape of the phenotypic frequency distribution will be conditioned by the properties of the individual polygenes, their dominance relations and interactions with each other. If, for example, in the simple digenic case discussed above **A** and **B** were completely dominant over their allelomorphs **a** and **b**, or if the **B**, **b** locus could only have an effect on the phenotypes of **aa** individuals, i.e. **A**, **a** was epistatic to **B**, **b**, the frequency distribution of phenotypes, as shown in Fig. 1, would no longer be symmetrical. It would be skew, with the mode to the left and long tail to the right of the figure. Skew distributions of this and the opposite kind, with its long tail to the left, are quite common, an example being afforded by plant height in barley (Fisher, Immer & Tedin, 1932).

Little, however, is known of those properties of polygenes which are responsible for skewness. Fisher (1930*a*) has advanced reasons for expecting polygenes often to show some dominance. Clearly dominance in polygenes, unlike dominance of the familiar laboratory oligogenes, cannot depend on an intrinsic advantage of one allelomorph over the other, for, as we have seen, neither allelomorph has such a regular advantage. Each allelomorph will have an advantage over the other in particular cases. Thus, if dominance is related to selective advantage, the − allelomorph, decreasing the degree of phenotypic manifestation of a character, is just as likely to be dominant as it is to be recessive to the + allelomorph which increases the manifestation. Fisher's argument leads to the conclusion that dominance of one allelomorph or the other will be determined solely by their relative frequencies in the population. We may then expect equal numbers of dominants in either direction. If, however, selection were to be so adjusted as steadily and regularly to favour a high or a low degree of expression, i.e. the + or − allelomorphs, the balance would be upset and dominance equality would no longer be encountered. In such cases the long tail of the phenotypic frequency distribution, which contains the recessive less frequent phenotypes, will point away from the direction of selection, and may be used to detect the direction of action of a selective force (Fisher *et al.* 1932).

These expectations are well supported by such data as are available at present. In man it has been shown that the frequency distribution for stature is symmetrical; yet, by the

use of correlation methods, which will detect dominance no matter what its direction may be, Fisher (1918) has found evidence of dominance of the polygenes controlling this character. Symmetry must then depend on an equal number of polygenes being dominant in either direction. This approach has been developed in more detail by Fisher *et al.* (1932) who introduced the use of third degree statistics. Taking data from Emerson & East (1913) these authors showed that the polygenes controlling plant height in maize, a character which shows heterosis, displayed dominance.

They also analysed data from a selection experiment with mice (Fortuyn, 1931). In unselected material the phenotypic frequency distribution was symmetrical, but selection in either direction introduced skewness with the long tail of the distribution towards the pre-selection mean, exactly as Fisher's view would lead us to expect.

Interaction of polygenes has been even less extensively analysed than their dominance. Two kinds of interaction, having very different genetical significance, can, however, be recognized. One gives rise to what Fisher terms metrical bias, the other being interaction of the epistasis type.

The first kind, leading to metrical bias, can perhaps best be understood by a consideration of Fig. 2, which shows a symmetrical frequency distribution. Now suppose that the scale of the abscissa was made logarithmic. What were originally equal intervals in Fig. 2 now become unequal and, in particular, the farther to the right we proceed along the abscissa, the greater is the shortening of the intervals. The effect of this will be to destroy the symmetry of the curve and render it skew. A similar result could be obtained by other transformations of the abscissa scale, as, for example, the square roots or cube roots of the abscissa values in Fig. 2. Skewness of the opposite kind would be obtained by such scalar transformations as using antilogs or squares.

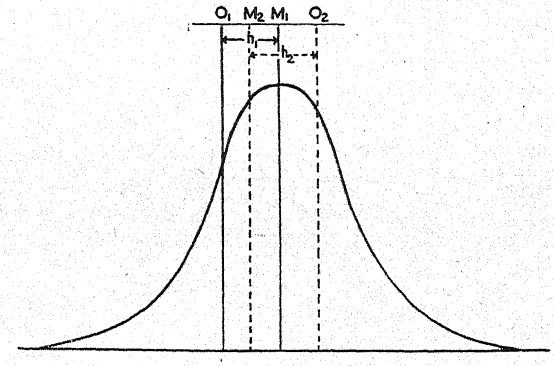

Fig. 2. The phenotypic distribution and optimum. The mean of the phenotypic distribution is M_1. When the optimum is at O_1, departing from the mean by h_1, the next generation has a mean lying between M_1 and O_1 at M_2. If, however, the environment is showing fluctuating change, the new optimum may be on the other side of M_1, at O_2. Then h_2, the departure of M_2 from O_2 will be greater than h_1. The selective response of the first generation has lowered fitness in the second. (The departures h_1 and h_2 are magnified for the sake of clarity.)

Now if the scale we find most convenient for measuring the character of the organism happens to bear the same relation to the unknown 'scale', on which the organism's physiological processes work, that, say, logarithms do to antilogs, or square roots to their generating numbers, skewness will be observed when the frequency distribution of the measurements is plotted. These kinds of skewness are described as metrical bias because they depend on the type of scale, or metric, used, and can be removed entirely by appropriate transformation.

No general theoretical significance can be attributed to metrical bias because, in the absence of external evidence, we have no right to assume that the scale we find convenient should bear any special relation to the processes on which depends the degree of expression of the phenotype in question. An understanding of metrical bias is, however, important

for the analysis of polygenic behaviour, because this bias can give rise to unexpected curves for inbreeding depression and other phenomena (Rasmusson, 1933).

Epistasis, and the related types of interaction shown, for example, by complementary genes, are theoretically quite distinct from metrical bias. The latter depends on the scale of measurement and will give a constant skewness, whereas the former results from the interdependence of genes in development. Thus complementary genes can produce no effect unless all are present. With two such genes (**A**, **a** and **B**, **b**) **aabb**, **Abb** and **aaB** are all alike, the distinct type being **AB**. Similarly when one gene (**A**, **a**) is epistatic to another (**B**, **b**), the latter will have no effect unless a given allelomorph of the former is present, e.g. **AB** and **Abb** will be alike though **aaB** and **aabb** differ. It is clear that no scalar transformation can regularly remove skewness of the phenotypic frequency distribution arising from this type of interaction, for such skewness will not be constant in magnitude. Nor is there any reason to expect this type of skewness to be characteristically in one direction. Rather, like dominance, it may give either positively or negatively skew distributions.

There is no certain evidence of the occurrence of polygenic interaction. Rasmusson (1935) has recorded a case in *Pisum*, but this example could equally be one of metrical bias. The same is true of Currence's (1938) findings in the tomato. Smith (1937) failed to find any evidence of interaction in the inheritance of corolla shape in *Nicotiana* species cross. Unless, and until, the contrary is shown to be the case, however, it is obviously necessary for caution's sake to suppose that any type of interaction shown by major mutants, i.e. oligogenes, may also be shown by polygenes.

Dominance, metrical bias and interaction can all cause skew phenotypic frequency distributions, and very extensive experiments would be required to separate their effects on any character. Such analyses have yet to be made, and so it is impossible at present to say how far the effects of the various agents are shown by the frequency distributions of polygenic characters.

These three agents may also have effects similar to one another on features other than skewness. In particular, all three will play their part in determining the relations which exist between the phenotypes of a hybrid and its parents. If, on the chosen scale, the genes are simply additive and show neither dominance nor interaction the hybrid phenotype will fall on the arithmetic mean of its parents; but this would also be the case where either dominance or interaction occurred, provided that it was equal in each direction. Lack of such equality or metrical bias, however, would destroy this simple relation. The observable relations between hybrid and parents would then depend on the degree to which dominance or interaction preponderated in one direction, or on the nature of the metrical bias.

Without extensive analyses of the features, and such analyses have never been made, no expectation can be formulated for the F_1 phenotype. It follows, then, that the discussion as to whether fruit size in the hybrid *Solanum lycopersicum* × *S. pimpinellifolium* falls on the arithmetic or geometric means of the parents must be sterile (MacArthur & Butler, 1938). The F_1 fruit size need not fall on either. Furthermore, even if one or other of these alternatives was observed in a particular case, this fact would by itself give no certain information about the nature of the polygenes involved. Any given relation may be obtained in several ways.

In just the same way the development of formulae (Charles & Smith, 1939) for

distinguishing between additive polygenes with dominance in one direction and a type of inheritance showing metrical bias without dominance is of very limited value. It leaves out of account many other possibilities, all equally likely a priori.

IV. THE STORAGE OF VARIABILITY

(1) *Fitness and flexibility*

Inasmuch as individuals vary in their phenotypic manifestation of a polygenic character, they may be expected to vary in the fitness, or relative selective advantage, which they enjoy in any given environment. The phenotype is, of course, subject to non-heritable as well as to heritable variation, but provided that heritable variation is present the mean degree of expression of any character in a progeny will be correlated with that of the parental phenotypes.

The magnitude of the regression of progeny on parent will be conditioned by the ratio of heritable to non-heritable variation, by the dominance, interaction, number and linkage of the polygenes and by the scale on which the phenotypes are measured; but whatever its exact value the regression must be positive. Big parents will produce big offspring and small parents small offspring. Hence any variation in selective advantage, consequent on variation in phenotype, is capable of causing the phenotypic frequency distribution of the next generation to differ from that of the parental generation. Such changes need not occur, however, where selective advantages are balanced against one another in the sense that every individual deviating in one direction from the centre of the parental distribution is matched, with regard to selective advantage, by an individual deviating in the opposite direction. A stable population is possible where the relative selective advantage diminishes as the phenotype departs from the centre of the distribution. The resulting tendency towards diminution in phenotypic variation is offset by the extra segregation from the centrally placed phenotypes, which, as will be seen from Fig. 1, are produced by the most highly heterozygous genotypes. Evidence of this correlation of selective advantage and departure from the mean phenotype has been obtained for recovery from rain damage and body form in sparrows by Bumpus and for longevity and shell radius in snails by Weldon (quoted by Baily, 1941).

Thus the mean expression of any phenotype, measured on a scale giving no metrical bias, must be the optimum phenotype if the population is to be stable. Otherwise selection would constantly be moving the mean towards the optimum (Mather, 1941). Actually, of course, the environment is itself never stable and hence mean and optimum will not exactly coincide. Every generation will give a progeny whose mean is nearer to its own optimum; but by the time the progeny has developed, the optimum will have changed and the process will be repeated (Fig. 2). Response to selection must always be a generation in arrears, because selective advantage is determined by the phenotype while selective response is expressed as a change in the daughter genotypes.

If the environment, and hence optimum phenotype, is changing steadily in one direction, the change which selection brings about in mean phenotype will be directly adaptive, in spite of the lag of one generation. But if the environment and optimum are subject to changes of a fluctuating kind, response by the organism is not adaptive. It is wasted, because the next generation is as likely as not to have a mean departing from the optimum in the direction opposite to the parental deviation. Since a persistent

response to selection must involve one allelomorph of at least one gene replacing the competing allelomorph (see § V (3)) selection destroys heritable variation; so undue reaction to these fluctuating changes in environment will, by diminishing heritable variability, reduce the chance of later changes by which the organism could adapt itself to environmental trends. Equally, however, if the heritable variability necessary for adaptation is present, response to fluctuating changes in the environment is inevitable. Adaptability and conservation of variability make conflicting calls on the organism.

There is a close parallel between this conflict and that between fitness and flexibility. Heritable variability is necessary for adaptive change, but, in that it implies some individuals departing from the optimum, it lowers present fitness; for, as we have seen above, departure from the optimum must be correlated with reduction in fitness. In the same way response to fluctuating environmental changes reduces the heritable variability upon which depends adaptation to environmental trends. The success of any organism, in competition with its contemporaries, must depend on the extent to which it reconciles these needs. Failure to achieve an adequate balance spells either its own doom, on the one hand, or that of its descendants, on the other. Existing organisms must therefore have descended from those which had most adequately balanced the advantages of fitness and flexibility in the past. The organisms of the future will equally be descended from those which, to-day, best reconcile the needs of fitness and flexibility, the rest dying out sooner or later.

(2) *Free and potential variability*

Immediate fitness requires that, phenotypically, all the individuals fall as near to the optimum as possible, i.e. that the phenotypic frequency distribution has a minimal variance. (The mean phenotypic variance of the progeny of an individual is, of course, bound to be highly correlated with that of the population as a whole.) But a complete absence of heritable variability would rule out future adaptation, except by mutation, and so doom the population to extinction. Such variability, which is the only adequate basis of future adaptation, need not, however, cause a marked lowering of present fitness, for it need not be phenotypic.

Except in special cases fitness is a property of the phenotype, which, apart from non-heritable causes, is controlled by the genotype acting as a whole. If a gene is completely dominant, the heterozygote **Aa** will be phenotypically like the homozygote **AA**, but, nevertheless, **Aa** individuals have a measure of heritable variability not possessed by the homozygotes. Such heterozygotes can give rise to **aa** offspring which, in general, will differ phenotypically from their parents and from their **Aa** and **AA** sibs. Some of the variability is not displayed phenotypically. It exists in the genotype in a hidden, or, as we shall call it, potential, state.

The free variability of a population is that which is manifested by the phenotypes. It will be open to the action of selection and, inasmuch as the various phenotypes differ in their degree of adaptation to the environment, it will inevitably be acted on by selection. The potential variation of a population is, on the other hand, not manifest in the phenotype and hence will be incapable of being affected in any direct way by selective forces. It will play its part in later generations when it has passed from the state of potential to free; just as the potential energy of a weight held in the air is freed when it is allowed to fall. Thus the conflict between fitness and flexibility can largely be

removed, for fitness is a function of the free phenotypic variability while flexibility largely depends on potential genotypical variability. Flexibility obviously will also depend in part on the free variability, but only to an extent proportional to the magnitude of free as compared with potential variability. The most advantageous arrangement will thus involve a small amount of the former and a large store of the latter. Inasmuch as the species of to-day are descended from the successful species of the past, their genetical structure must betray the means by which this balance of free and potential variability is achieved and maintained.

Before passing to a discussion of how storage of variability can be effected it must be made clear that no amount of potential variability is of use unless it can be freed. Future adaptation depends on the interaction of free variability with selection. Furthermore, as trend changes in the environment must be presumed to be always in progress, even though masked at any moment by fluctuation changes, a quantity of free variability must be available in every generation. In other words, there must be a steady flow of variability from potential to free. The storage mechanism must provide for a gradual leakage.

It is also clear that unfixed free variability must pass back into store sooner or later, for otherwise the amount of free variability would increase from generation to generation. This cycle of variability change, from potential to free and back, may be interrupted by selective forces which fix some of the free variability, so causing selective response. This fixation will only occur with such variability as is appropriate to the selective force, which may thus appear to create its own directional variability (see 'Student', 1934). In truth, however, the discriminative action of selection is solely one of fixing and so rendering visible variability which would otherwise pass back into store. Selection cannot create new variability.

(3) *Linkage and the storage of variability*

The variability of a population will be wholly free when all the individuals have completely homozygous genotypes giving the maximum phenotypic departure from the mean possible with the available genes. If this condition is not fulfilled some of the variability must be in store. Thus with a single gene the variability will be wholly free when all individuals are either **AA** or **aa** (except in the rare and complex case of **Aa** being more extreme than either homozygote). If **Aa** individuals are present some of the variation is potential, for interbreeding these heterozygotes will give phenotypically detectable segregation in the next generation. The progeny will then show phenotypic variation not possessed by the parents. So we may recognize one way in which variability can be stored, viz. by heterozygosity, and the way in which it is released, viz. by segregation. We may also note that, since intercrossing **AA** and **aa** individuals produces **Aa** heterozygotes, crossing is the means of converting free into potential variability.

When two or more genes affect the same character, a further type of storage becomes possible. Consider two non-interacting genes **A**, **a** and **B**, **b** where the effects of **A** and **B** differ in a similar way from those of **a** and **b** respectively. The extreme homozygotes are **AABB** and **aabb**, and providing no other gene is involved the variability will be wholly free when all individuals fall into one or other of these classes. The various heterozygotes, **Aabb**, **aaBb**, **AaBb**, **AaBB** and **AABb** will show some storage of variability, but we have also to consider the remaining homozygous types **AAbb** and **aaBB**. Here the two genes are acting in opposite directions, the phenotypes being, in consequence,

intermediate between those of **AABB** and **aabb** individuals. Hence some variability must be stored by these homozygotes. Such homozygotic variability cannot be released directly. It must always first be converted, by intercrossing, into heterozygotic variability whereupon release by segregation becomes possible (Fig. 3).

With more than two genes affecting the character this type of homozygotic storage becomes increasingly important because homozygous genotypes giving phenotypes of various degrees of intermediacy may occur. Thus the ratio of free to potential variability of a polygenic character is very flexible. All values are possible.

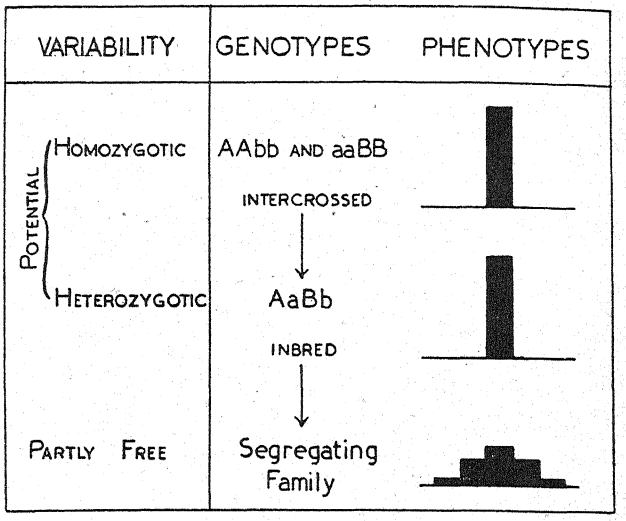

Fig. 3. The release of potential variability. Potential variability is released by segregation. Homozygotic potential variability cannot be released directly. It must first be converted by crossing into heterozygotic variability.

When the character is affected by two or more genes of similar effect, the release of variation will depend on recombination, and hence will be affected by linkage (Mather, 1942*b*). Continuing the two-gene example of the preceding paragraph, let **Aa** and **Bb** be intermediate between **AA** and **aa**, and **BB** and **bb** respectively, and let the two genes have equal additive effects. On selfing or interbreeding double heterozygotes, **AaBb**, the F_2 will contain the ten possible genotypes with the following frequencies, p being the recombination value, and $q = 1 - p$.

Coupling **AB**/**ab**

	AA	**Aa**	**aa**
BB	q^2	$2pq$	p^2
Bb	$2pq$	$\frac{R\ 2p^2}{C\ 2q^2}$	$2pq$
bb	p^2	$2pq$	q^2

Repulsion **Ab**/**aB**

	AA	**Aa**	**aa**
BB	p^2	$2pq$	q^2
Bb	$2pq$	$\frac{R\ 2q^2}{C\ 2p^2}$	$2pq$
bb	q^2	$2pq$	p^2

R indicates the repulsion and C the coupling double heterozygotes, **Ab**/**aB** and **AB**/**ab**. The phenotypic expression of the character may be measured by the number of capital letters in the genotypes. **AABB** gives a phenotypic expression of 4, **AaBB** and **AABb** one of 3, **AAbb**, **AaBb** (both C and R classes) and **aaBB** 2, **aaBb** and **Aabb** 1 and **aabb** 0 (see Fig. 1).

Fig. 4 shows the phenotypic frequency distributions obtained with various values of p in coupling and repulsion, and it will be seen that the distribution depends on the linkage conditions. The quantity h is the amount of free variability in the progeny expressed as

a fraction of the total variability. Now the parents, **AaBb**, were the same for every progeny, and show no free variability. All the variability was potential, and hence the differences in free variability shown in the next generation depend on differences in the rate of release of this potential variability. Rate of release is thus clearly modified by the linkage conditions. Tight linkage in repulsion, i.e. where the two genes have allelomorphs of like effect in different chromosomes, will give slow release, and tight linkage in coupling, i.e. where allelomorphs of like effect are in the same chromosome, quick release, loose linkage in either phase giving intermediate results. As the frequency of recombination between two genes in the same chromosome is capable of modification by selection (Detlefsen & Roberts, 1921) and by inversion of chromosome segments (Darlington, 1937) the rate of release can be adapted to an optimum value. The freedom of recombination of whole chromosomes can also be changed by reciprocal translocation, leading to ring formation; but this method would appear to offer greater difficulty and be less widespread than control of recombination within chromosomes. Hence it is to intra-chromosome adjustment that we must look for the storage and control of variability.

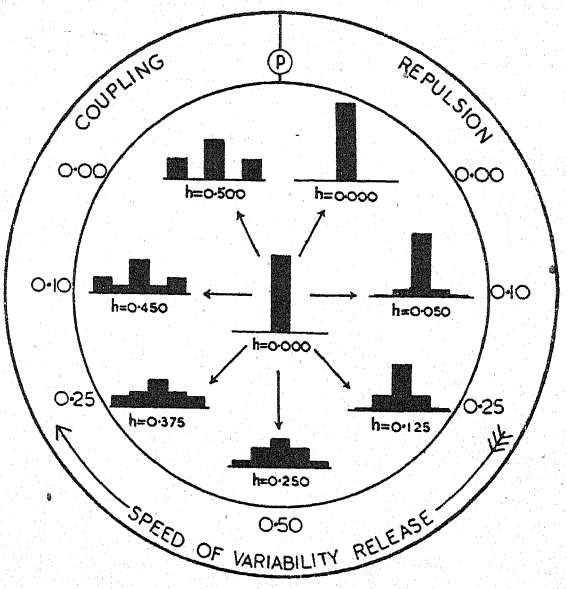

Fig. 4. Recombination and variability release. Starting with individuals heterozygous for two genes (**AaBb**), all the variability being thus potential, interbreeding releases some variability by segregation. The actual amount released is governed by the linkage relations of the two genes. With low frequency of recombination (p), release is slow in repulsion, and fast in coupling. Loose linkage gives intermediate release. The parental phenotypic distribution is shown in the centre, those of the next generation, assuming various recombination frequencies, being immediately round it. h is the fraction of the variability which is released. Its maximum value of 0·5 is achieved in close coupling (see text).

Storage may be either homozygotic or heterozygotic, release depending ultimately on segregation in either case. When two or more genes affect the character, and with polygenic characters many genes are involved, recombination frequency controls the rate of release, the main control being exercised within chromosomes. Segregation of any gene is not itself influenced by that of the others, but linkage determines the frequency with which the phenotypic effect of segregation in one gene is reinforced or nullified by that of the others. It determines whether segregation will release the variability or merely recast it into a slightly different potential form. Intercrossing controls the flow of homozygotic to heterozygotic store, and also of free back to the potential state.

(4) *Balanced polygenic combinations*

Linkage can, according to its phase, either speed up or slow down the release of polygenic variability, but if a slow rate of release is determined by close linkage in the repulsion phase, very rapid release must occur when the coupling phase supervenes.

Such a system would show sharp alternation between slow and rapid release. This clearly has serious drawbacks for the organism, for if slow release is advantageous, the periods when the coupling phase dominated would be markedly disadvantageous. Two factors help, however, in mitigating this consequence.

In the first place, distinctly more than two of the polygenes affecting a given character may be expected to lie in any chromosome. A very complex series of linkage types will then be possible. With two genes only two fully heterozygous zygotes are possible, **AB/ab** and **Ab/aB**. The number rises to four with three genes, **ABC/abc**, **ABc/abC**, **Abc/aBC**, **AbC/aBc**, and to eight with four genes, **ABCD/abcd**, **ABCd/abcD**, **ABcD/abCd**, **ABcd/abCD**, **AbCD/aBcd**, **AbcD/aBCd**, **AbCd/aBcD**, **Abcd/aBCD**. It is increasing according to the rule 2^{n-1}, where n is the number of linked genes. Now certain of these multiple heterozygotes are complex mixtures of coupling and repulsion. Thus in **AbCd/aBcD**, two pairs of genes are coupled internally but repulsed with respect to each other. The most that a single cross-over can do in such a case is to bring three genes into the coupling phase, the fourth being in repulsion. The change in phasic balance here is not great, and with more genes still less drastic changes may be expected to follow single recombination. Polygenic behaviour has a statistical stability not shown by single genes, for the same reason that a volume of gas has predictable properties though the behaviour of a single molecule is quite unpredictable.

This conclusion will be true even if the arrangements in the chromosomes occur with random frequencies. But the second agent making for stability enters in at this point. There must be a selection for the more stable arrangements, i.e. for those in which the phasic balance is least upset by recombination.

We have already seen that the rates of release in repulsion and coupling are negatively correlated. In other words, the greater the advantage of repulsion, the greater the disadvantage of coupling. So a drastic change in phasic balance must ultimately be followed by a marked lowering of fitness and, in consequence, stable phasic types will carry a selective advantage and will tend to oust their competitors. The less advantageous types will not of course disappear, for they will continually arise as recombination products from the favoured arrangements. An equilibrium will exist between selective increase and recombinant decrease in favoured types and between selective decrease and recombinant increase in deleterious arrangements. We can thus recognize still another characteristic of polygenic characters. A population may show a constant phenotypic frequency distribution and a constant mass breeding behaviour, but this is the outcome of a complex of balancing processes causing a steady reshuffling of the genetic material in the very individuals which in the aggregate show this statistical constancy. Constancy of the phenotypic distribution in a constant environment is produced by the very agents on which depends selective change in a changing environment. Selection is necessary for stability, and, as Haldane (1936) points out, this stabilizing action of selection makes much more plausible its role as the agent of evolutionary change.

In the general case we may expect the free variability of a population to be low and the potential variability high. Hence neighbouring polygenes in a chromosome should be in repulsion, i.e. have opposing tendencies. Otherwise the rate of release will be high and variability will be diminished to a dangerous extent by response to fluctuating changes of the environment. The desirable balance of fitness and flexibility would then be lost. An arrangement which maintains the optimum, or near optimum, balance of fitness and

flexibility will be referred to as a balanced polygenic combination, and is characterized by the twin properties of having a phenotypic effect near to the optimum for the constituent polygenes, and of releasing its variability only slowly by recombination with other homologous combinations of the same chromosome. The further the phenotypic effect is from the optimum and the more easily the combination is broken up, the poorer is its balance. A well balanced combination causes little phenotypic variability while maintaining, in relation to available homologous combinations, the possibility of great ultimate change slowly produced.

Selection experiments have shown that such combinations exist in *Drosophila melanogaster* (Mather, 1941; Wigan, 1942; Sismanidis, 1942) and they can be inferred from Winter's (1929) results with maize. The great storage capacity is well brought out by Payne's (1918) selection for increase in the number of scutellar bristles in *Drosophila*. In both wild and stock flies this number seldom varies from 4, but a fly with 5 is occasionally found. Starting with a 5 bristled female and a 4 bristled male, Payne raised the mean number to about 6·5 in eleven generations, and to over 9 in thirty generations. Sismanidis (1942) has confirmed Payne's results and has been able to assign the sudden responses to selection, to recombinations in particular chromosomes (Fig. 5).

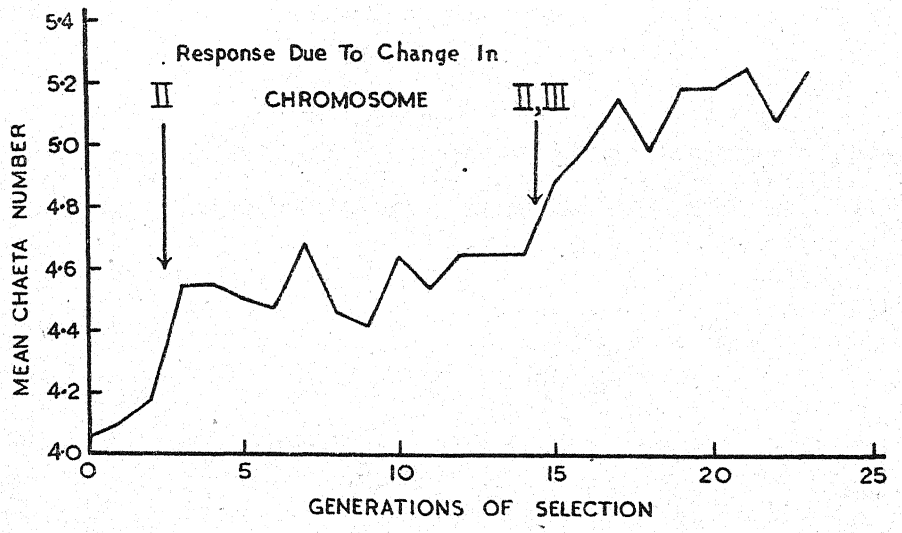

Fig. 5. Recombination and selective response. The result of a selection experiment for increased number of scutellar chaetae in *Drosophila melanogaster*. The response occurs in two rapid steps, with intervening stability. Tests show that these steps are separable as being due to changes, presumably recombinations, in distinct and recognizable chromosomes. (Reproduced by kind permission of Dr A. Sismanidis.)

V. THE PROPERTIES OF POLYGENIC COMBINATIONS

(1) *Internal and relational balance*

The two features which determine the relations between free and potential variability are the aggregate recombination frequency and the arrangements of the polygenes in the chromosomes. Darlington (1939) has shown how completely genetic systems and their evolution are interpretable in terms of the control of recombination. We can now see why recombination is so important. It is the tap controlling the flow of polygenic variability.

We can see also how the theory of polygenic inheritance aids us in understanding the selective control of recombinations. Fisher (1930*a*) has shown that there is a general selective advantage in tight linkage between two genes, provided that their combinations enjoy constant advantages relative to each other. But we have seen that polygenic inheritance is typified by inconstancy of such advantage. Consequently the situation here is very different. Just as the relative advantage of the allelomorphs of a given polygene vary with the other polygenes present, in that these others can enhance or counteract the effect of the gene in question, the advantage of a given combination of any two genes will depend on the constitution of the individual for the other polygenes affecting the character in question. Though in one set of individuals **AB** and **ab** may be more favoured than **Ab** or **aB**, the reverse may be true elsewhere and, as recombination is the only means short of mutation of changing the arrangement, this inconstancy of advantage must favour some degree of recombination.

Secondly, Sturtevant & Mather (1938), in listing the conditions necessary for the selective favouring of recombinations between two genes, found it necessary to stress that the genes must be maintained heterozygous in order that the recombination be effective. They were forced to complicate the conditions to this end. It is clear, however, that with systems of many polygenes some of them, though not necessarily the same ones, will always be heterozygous. There will be a statistical constancy of the proportions homozygous and heterozygous at different times. Consequently, as recombination must affect all gene arrangements, Sturtevant & Mather's special condition is not essential. Its necessity vanishes when we cease to think in terms of a minimal number of genes.

Turning to the second agency affecting storage and release, viz. the genic arrangement, two types of polygenic balance must be recognized (Mather, 1941). (It should be made clear that we are referring to the balance within each chromosome. As we have seen above, different chromosomes usually recombine freely and hence each must achieve its own balance independently of the rest, the control of variability being achieved almost entirely by intra-chromosome adjustment.) In an inbreeding organism, such as wheat (Mather, 1940) or the grass mite *Pediculopsis* (Cooper, 1937), individuals homozygous for one or more chromosomes must occur freely. If such individuals are fit, their polygenic combinations must, when homozygous, have an effect near to the optimum (Fig. 6). The combinations must each be internally balanced, and natural selection will in fact favour the occurrence of such a balance in inbreeding organisms.

If, however, we consider an outbreeding organism, such as maize, it is clear that heterozygosity of chromosomes will be the rule. The internal balance of a combination is then of little importance for survival, being overshadowed by the relational balance existing between pairs of different homologous combinations. In such cases natural selection will tend to build up relationally balanced combinations. We can see the consequence of this in maize, which when inbred gives homozygous types poor and feeble in comparison with the highly heterozygous individuals of an open-pollinated variety (Fig. 6). The internal balance displayed in the homozygotes is poor, but the relational balance shown in heterozygotes is good. We may note that the existence of poor internal combined with good relational balance implies dominance of polygenes, as otherwise the heterozygote would always be intermediate between the homozygotes, and the types of balance would not be capable of separate adjustment.

Adaptive relational balance supplies the key to a number of problems of equilibrium.

It shows us, for example, how polymorphism can be maintained (Mather, 1941). If the internal balance is poor, homozygotes are less fit than heterozygotes; and when each combination is completely linked, as by means of an inversion, with a major oligogenic mutant or marker gene the polymorphic system, found for example in grouse locusts, (Fisher, 1930*b*, 1939) is complete. The consistent advantage of all the many heterozygotes over all the homozygotes, as observed in these cases, is not easy to understand except in terms of polygenic combinations.

The relative advantage of any combination depends on those which accompany it, and so we might expect the relative frequency of occurrence of any one to vary with external conditions. Any major mutant inseparably linked with a given combination would vary correspondingly in frequency and give a 'cline' (Huxley, 1939). Such a cline would be

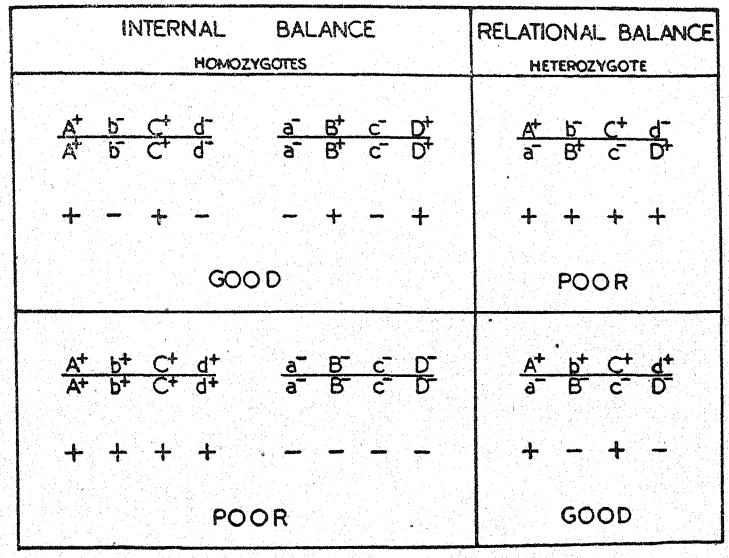

Fig. 6. Internal and relational balance of polygenic combinations. Capital letters indicate dominant and small letters recessive allelomorphs. The indices, + and −, show the direction of action of the allelomorphs, the sum effect of the allelomorphs at any one locus being shown by the + or − below. The optimum is assumed to be two +'s and two −'s, any departure from this being taken as indicating unbalance. Dominance permits the two balances to be varied independently of one another. Note that the case of poor relational balance is also one of heterosis.

stable unless conditions are changing rapidly in any place. Polygenic theory allows us to see how equilibria are maintained, because it shows us how optimum phenotypes depend on harmonious combinations of polygenes rather than on the properties of the individual units of genotypic variation. The statistical, and hence relatively constant, properties of the aggregate hide the vagaries of the individual gene.

(2) *Heterosis and the origin of isolation mechanisms*

Heterosis is the name usually applied to the phenomenon of increase in vigour sometimes observed when strains of an organism are intercrossed (Darwin, 1876). This extra vigour is more apparent in cross-breeding organisms if the parents are themselves highly inbred and hence poor types. Heterosis should, however, be measured in such cases by the excess of the hybrids over the average of the open breeding varieties from which the

parental homozygotes had been derived. In this way the distracting effect of inbreeding depression is avoided.

In crop plants, especially maize, heterosis is advantageous from the cultivator's point of view, for it allows him to obtain heavier or earlier crops. In nature, however, the situation must be very different.

Intercrossing two strains results in bringing together unlike polygenic combinations in the hybrid. If the histories of the two strains are separate, the two combinations received by the hybrid will not previously have been together and hence will not have been selected for good relational balance. The phenotype of the hybrid will be likely to show a greater departure from the optimum than does either parental strain, because combinations within the same strain will have been selected for relational balance. The departure of the hybrids from the optimum, or optima, to which the parental strains are adapted, will, if in the direction of increased size, be hybrid vigour or heterosis. We can thus recognize that in nature heterosis is a sign of poor adaptation and must be selectively disadvantageous. Hence, inasmuch as all departures from the optimum will be disadvantageous, whatever their direction (§ IV (1)), the concept of heterosis may properly be extended to include all examples of poor relational balance between combinations of different wild interbreeding groups (Fig. 7).

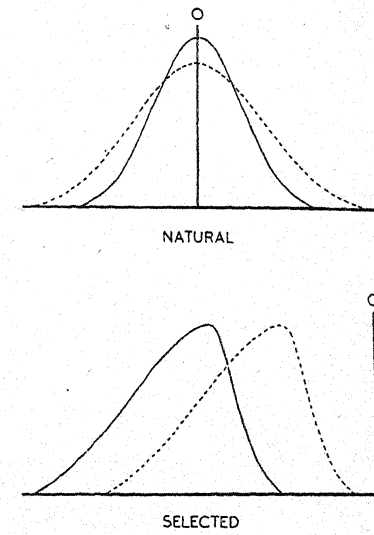

Fig. 7. Heterosis. In a wild organism the phenotype varies round the optimum, *O*, without any dominance bias. Heterosis in natural populations is shown by the phenotypic distribution of the progeny (dotted line) having a greater spread than that of the parents (solid line). Artificial selection may be regarded as moving the optimum outside the range of the phenotypes. The distribution then shows dominance bias and, in consequence, heterosis causes the progeny distribution to approach the optimum more nearly than that of the parents (see text).

Now if two strains or populations breed less freely with each other than either does within itself, selection will have less opportunity of maintaining the relational balance between combinations of different strains than between those of like strain. The genotype being fluid, even though the phenotype is constant (§ IV (4)), relational balance will deteriorate unless constantly maintained by selection. Hence heterosis in our new sense is an automatic consequence of any diminution in the freedom of interbreeding between strains. Now as heterotic individuals will be less fit than the parental types, their production represents wastage of reproductive effort by the parents, and any variants tending to reduce outbreeding between the strains will be favoured. In this way isolating mechanisms (Dobzhansky, 1941) and hybrid sterility originate. It is to be presumed that both isolation mechanisms and hybrid sterility are polygenic characters capable of developing from the species, store of variability. They will then be inevitable results of heterosis. Other special conditions may have the same result (Sturtevant, 1938), but the inevitability of this polygenic effect makes it seem likely that the avoidance of heterosis is the most widespread stimulant of isolating devices.

Such a system must be self-propagating, because the less the intercrossing which goes on between strains, the greater is the chance of unbalance and heterosis, and the

stronger is the advantage of isolation. Thus a small decrease in mating freedom, such as may be brought about by natural obstacles, slightly different conditions between adjacent localities, or a change in the breeding system (§ VI (3)) will suffice to start the chain of events, which once in progress cannot be reversed. Once a complete isolation mechanism is achieved and crossing prevented, the two strains, now species, can invade each other's territory and all evidence of how they came to be separated will be lost. This process now appears to be nearing completion in *Drosophila pseudo-obscura*.

Heterosis, like the simultaneous occurrence of good relational and poor internal balance, implies dominance of polygenes (cf. Jones, 1917). In § III (2) we have given evidence that in natural populations dominance is not directional. The + allelomorph is as likely, but no more likely, to be dominant as recessive to the − allelomorph. This lack of direction is a consequence of the variation of the phenotype round its optimum. Hence if the optimum were maintained outside the phenotypic range, dominance of that allelomorph making for a phenotype nearer to the optimum would become more frequent than the reverse. So polygenic characters, such as yield in crop plants, where the breeder always selects in one direction, should show dominance preponderantly in the direction of selection, with the result that disturbed relational balance should most often cause hybrids to depart from the strain means in the direction of previous selection. Dominance bias and heterosis in crops should be directional (Fig. 7).

Hutchinson, Panse & Govande (1939) have found this to be the case in cotton, wherever the character was one of commercial importance, and hence presumably the subject of artificial selection; but with unselected characters of no commercial interest, no such directional departure was observed. In their sense of the word, no heterosis was found.

Evidence of the undirectional nature of heterosis, in the present sense, in organisms not artificially selected, is provided by Sveshnikova's (1935) observations on *Vicia cracca* where interstrain hybrids were often poor, by East (1935) in species hybrids of *Nicotiana* and *Fragaria*, and by unpublished observations which my colleague Mr W. J. C. Lawrence has allowed me to quote. He finds that species hybrids of *Streptocarpus* are of very variable vigour but do not regularly depart in one direction from their parents. Crosses of these species with the artificially selected garden forms, however, give hybrids which more often than not show increased vigour.

(3) *Mutation and the maintenance of variability*

The heritable variability of a population is constantly being reduced in two ways (Mather & Wigan, 1942). One of these is by the random elimination of allelomorphs. Such elimination is a consequence of the fact that more gametes are produced by the zygotes of one generation than will be represented in the zygotes of the next generation. Sampling leads to random changes in the allelomorph frequencies and will eliminate allelomorphs with a frequency inversely proportional to population size (Fisher, 1930*a*; Wright, 1940).

The second way is by selection. Selection can act as a stabilizing agent of the allelomorph frequencies, as in cases of good relational combined with poor internal balance. But if selection produces a permanent adaptive or evolutionary change in the organism, it must do so by changing the genotype, i.e. by an allelomorph completely replacing its competitors at one or more loci. Heritable variability will thereby be reduced. Selection will destroy the very variability by virtue of which it was effective.

Clearly there must be an agent counterbalancing both random and selective extinction,

and it is to be found in mutation. Polygenes mutate just as do oligogenes, but the rate of accumulation of polygenic variation is slow. Johannsen (1909) with beans, Lindstrom (1941) with tomatoes, and others, found little evidence of polygenic mutation, but Mather & Wigan (1942) were able to observe the results of mutation during fifty-three generations of selection in *Drosophila melanogaster*. The new heritable variability was not immediately available to selection, as its small effect was masked and protected by non-heritable fluctuation. In time, however, recombination brought together mutant genes to give variants large enough for selection to act on in spite of fluctuations. Selective advances then occurred.

The rate of origin of new variability was so slow that after twenty generations the effect of selection was still small as compared with that obtained in ten generations' selection of hybrid material (Mather, 1941; Wigan, 1942). Thus, unless highly inbred, an organism's response to natural selection must depend almost entirely on the utilization of stored variability. Mutation mainly serves to replenish the store and so compensate for the small regular loss by random and selective extinction (Fig. 8). A little of the new variability will be, of course, free from its time of origin, for, mutation being an undirected process, some must arise in combinations which thereby become slightly unbalanced. Most, however, passes into store.

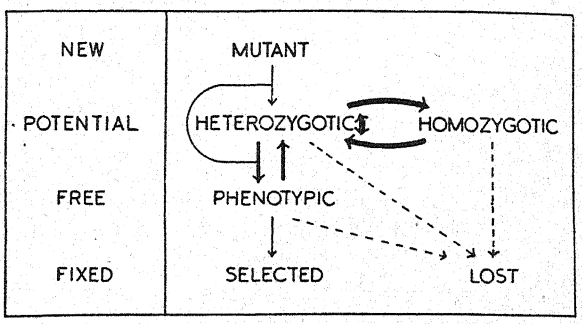

Fig. 8. The states of polygenic variability. New variability arises by mutation, most of it going into store, though a small amount is immediately free. Most free variability is derived from the potential store by segregation, and most of it returns to store by crossing. Some free variability is however fixed by response to selection, and some, like part of the potential variability, is lost by random fluctuation of the allelomorph frequencies. The potential variability is in a continual state of change from heterozygotic to homozygotic and vice versa, and also from one heterozygotic state to another. The relative magnitudes of the various normal changes are indicated by thickness of the solid arrows, the actual magnitudes being governed by the recombination frequencies and the linkage organization. The dotted arrows mark changes due to random fluctuations dependent for magnitude on the effective breeding size of the population.

This steady flow of new variability into store, and the equally steady release from and return to store of free variability, is possible only with polygenic characters. The new variability may be due to mutation of polygenes other than those which are being fixed by selection, but, since all like polygenes have similar effects, replacement of variability can be effected by genes other than those which are lost. It is impossible to say which polygenes will exist in the population as two or more allelomorphs, and which will not be varying at any given moment; but it can be said that a characteristic proportion will be in each state. It is on the fact that such a proportion exists in the heterogeneous state that variability depends.

The genotype is constantly changing as new mutants arise and old ones are fixed or lost. Variation is passing into store and is also being released (Fig. 8). Even inside the polygenic combinations there is a constant reshuffling, as new ones are formed and old ones destroyed by recombination. Selection is causing some to increase and others to decrease in frequency (Mather, 1942*b*). Yet, in spite of all this, the phenotypic distribution can be nearly constant, unless the environment is changing rapidly. Just as polygenetics resolves the contradiction between discontinuous genotypic and apparently

continuous phenotypic variation, it resolves the contradiction between genotypic flux and constancy, or near constancy of the phenotypic distribution.

The importance of recombination is that its frequency regulates the rate of all these transformations of variability (Fig. 8). Low recombination gives stability and high recombination gives flux. In *Drosophila*, where recombination is low (Darlington, 1934*b*), stability is upset only by rare, and hence very obvious, changes (Mather, 1941). The flux is small as might perhaps be expected in an organism whose successive generations encounter seasonally variable environmental conditions. In mice recombination is much higher (Crew & Koller, 1932). Here the release of variability, and hence the response to artificial selection, is easier and steadier (Goodale, 1938). Maize is intermediate both in recombination frequency (Darlington, 1934*a*) and response to selection (Winter, 1929).

(4) *Correlated response*

So far selective response has been discussed, for simplicity, in terms of a single polygenic character, to whose departure from the optimum phenotype loss of fitness was equated. In truth, however, fitness must be dependent on, and compounded of, many such polygenic characters, and response to natural selection must also be compound. The response in one character will depend on the correlated selection of other characters (Anderson, 1939).

Correlated response may happen in two ways. First of all, each polygene may be pleiotropic and simultaneously affect several characters. In such cases the correlation cannot be broken, and the two characters may be treated as one.

The second kind of correlation is mechanical. Polygenic combinations affecting two characters may occur in the same chromosome, and their constituent polygenes will then be intermingled. Recombination which changes the balance of one will usually, though not of necessity always, unbalance the other. The two characters will generally show simultaneous release of variability. So even though the phenotypic variants for one character, made possible by the recombination, might enjoy a selective advantage in appropriate conditions, the correlated unbalance of the second combination, intermingled with the first, must often tend to nullify this advantage, as conditions are not likely to favour both variants simultaneously. The result will be a slowing down of changes in the hereditary constitution of the population undergoing selection; but the changes will generally be possible in the end as, sooner or later, recombination will, step by step, re-order the intermingled combinations until something approaching the optimum is obtained. Response of the first character then becomes possible without a deleterious effect on the second.

In special circumstances, however, events may follow a different course. The rise in selective advantage of changed manifestation in the first character may be due to conditions which reduce the disadvantage of variation in the second one. The balance of advantage will then be towards change in both, and the second character will degenerate by virtue of the mechanical relations of its polygenes with those of the first. This has perhaps occurred for example in cave animals as Professor R. A. Fisher has pointed out to me. The advantage of good sight ceases on migration into the cave, while new adaptation of, for example, touch is favoured. The mechanical relationship of the two sets of controlling polygenes in the chromosome would be sufficient to make degeneration of the eyes an inevitable accompaniment of the rise of these new adaptations.

VI. BREEDING SYSTEMS AND THE CONTROL OF VARIABILITY

(1) *The adaptive nature of breeding systems*

The control of variability, its storage and release, depends chiefly on the linkage relations of the polygenes within chromosomes. The frequency of recombination determines the rate of exchange between the various states of variability. But segregation cannot occur and recombination is ineffective unless the organism is heterozygous for at least some of its polygenes. With complete homozygosity no potential variability can be released and, apart from some mutation, the free variability must steadily diminish. A homozygous system is static and, though perhaps showing high present fitness, is doomed to ultimate extinction by its inflexibility. Heterozygosity, on the other hand, sacrifices some fitness to the maintenance of flexibility. Genes which control the rate of outcrossing, or breeding system, control the balance of homo- and heterozygosity, and so will have an adaptive value in determining the variability transformations of the population (Mather & de Winton, 1941).

Genetical control of breeding systems is well known. Most animals show sex separation, while in plants there is a wide variety of mechanisms ranging from physiological incompatibility to special pollination structures. Some of them, like sex separation, incompatibility of the *Nicotiana* type (East, 1929) and heterostyly (Mather & de Winton, 1941), are recognizable as highly efficient means of promoting outbreeding and heterozygosity. These systems depend on genetical heterogeneity, matings between like types being prevented. Other outbreeding mechanisms, such as protandry and floral devices for attracting insects, do not depend on heterogeneity since all individuals show the same basic behaviour. They do not usually serve to prevent self-fertilization so completely and hence are of lower efficiency. Inbreeding mechanisms, like premature anthesis in *Triticum* and intra-uterine copulation in *Pediculopsis*, also exist and encourage homozygosity. These never depend on genetical heterogeneity.

Morphologically and physiologically these means of controlling breeding are very varied, especially in plants. (Most botanical textbooks, e.g. Kerner & Oliver (1894–5), devote large sections to their description.) The exact nature of the system depends on the morphological and physiological possibilities of the species (Mather, 1940, 1942*a*). Sex separation, for example, is well suited to mobile organisms and is less efficient in sessile forms (see Lewis, 1942*a**), which, however, may be able to develop a highly efficient incompatibility system.

From the point of view of function, breeding systems are classifiable on a two-dimensional basis. Some are more efficient than others in the control they exercise, in that chance plays a less part in determining the mate. Thus incompatibility and sex separation can completely prevent self-fertilization, whereas protandry and protogyny only reduce the chance of this happening in the place where it is most likely to occur, viz. within a flower. The former are more efficient than the latter.

The second dimension is provided by the rate of outbreeding, or, speaking inversely, of inbreeding. Incompatibility and heterostyly lead to outbreeding, homostyly and premature anthesis to inbreeding. This dimension must be carefully distinguished from the first one. Inefficient control will permit mixed breeding, but a superficially similar result can follow from a carefully controlled system. The latter, however, will enforce rather than permit mixed breeding, the average degree of outbreeding being thus less

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subject to chance variation than that resulting from poor control. Balanced controlled systems are achievable by mixed outbreeding and inbreeding systems of kinds dependent on the nature of the organism. Incompatibility systems may have a fertility allelomorph (East, 1929) whose frequency determines the balance of self and cross fertilization. Heterostyled species may include homostyled individuals (Crosby, 1940) giving a measure of inbreeding.

With sex separation the method of balancing outbreeding and inbreeding may be different again. The efficiency of sex separation, even in a mobile organism, depends on the ability of one sex to recognize individuals of the other sex for the purpose of mating. Control of the degree of outbreeding can be established by an extension of this discrimination. If individuals not only recognize those of the other sex, but can also distinguish particular groups within that sex, preferential mating will occur. Where discrimination is against individuals from the same population, the rigour of outbreeding is increased and where mating is preferentially within the population, inbreeding is increased. Evidence of such discrimination, or sexual selection, has been obtained both between and within species from observations on *Drosophila miranda* (Dobzhansky & Koller, 1938; see also Dobzhansky, 1941). Sometimes the control of breeding may, as with gynodioecy, lead directly to a balanced system (Lewis, 1941).

It is clearly not to be supposed that the balance of inbreeding and outbreeding observed in any given existing organism represents the present optimum, because, obviously, the present optimum depends on the degree to which change in the future will be advantageous. The existing balance must be one which has been sufficiently close to some past optimum for the ancestor of the present species to survive, and perhaps spread, while competitors, not possessing such a good balance, were extinguished. Such a balance will be of advantage to existing organisms only to the extent that future changes of environment match past changes. The survival of any present day species will depend on the extent to which its breeding balance agrees by chance with, or can be adapted to, an optimum, or series of optima, which only the future can decide. Thus most existing organisms will fail and must perish. Existing breeding systems do, however, show us how future adaptation of the breeding system can occur, for they show us how it has occurred in the past.

(2) *The genetical control of breeding*

The regular control of breeding can only be achieved genetically, because, otherwise, the controlling mechanism could not be a permanent feature of the species. The rise of breeding control must, however, be gradual, for two reasons. First of all the spontaneous origin of a highly specialized and complex mechanism is extremely unlikely, and, secondly, it is also very unlikely that the conditions determining the advantage of a given rate of outbreeding will either arise suddenly or persist indefinitely. (The rate of environmental change in the glacial period was very different from that of the Pliocene.) The development of control must be by small steps, and any subsequent change in the control must be equally gradual. So we should expect the controlling mechanism to be polygenic. If this be granted, it is easy to see that any organism is capable, under the action of natural selection, of developing and of changing its breeding system, by virtue of the store of polygenic variability with which it is endowed.

The genetical study of breeding control is still in its infancy, but so far nothing has been found to contradict this expectation, and indeed there is a certain amount of

evidence in its favour. The homogeneous breeding systems are uninvestigated, but appear to offer no difficulties. In almost all cases the special arrangement of flowers, or the timing of anthesis relative to receptiveness of stigmata, is obviously a quantitative character of the type which we may confidently expect to be polygenic.

On the face of things, heterogeneous systems of control are less amenable to a polygenic interpretation, in that they depend on 'switch' genes determining the classes within which mating is impossible or unlikely. But this is only part of the mechanism, for the strength of the mating reaction, controlled by the switch genes, may itself vary. In the heterostyled *Primula obconica*, for example, illegitimate matings, between pin and pin or thrum and thrum, never set more than 10% of the seeds obtained from the legitimate matings, pin by thrum and thrum by pin (Lewis, 1942*b* and unpublished), while in *Primula sinensis* the ratio may be as high as 70% (Lewis, 1942*b*; Mather & de Winton, 1941). Darwin (1877) gives other data of the same kind for a number of plants. The strength of the incompatibility reaction varies similarly in hybrids of the incompatible *Petunia integrifolia* and the compatible *P. axillaris* (Mather, unpublished). Sex in *Lebistes* (Winge, 1934) and *Apocheilus* (Aida, 1936) is also variable independently of the direct control by *X* and *Y* chromosomes. Thus the evidence suggests that this control of reaction strength is polygenic in *Primula*, *Petunia* and most cases of sex separation, though in *Lymantria* Goldschmidt (1933) thinks that it is not so. Winge (1937), however, considers Goldschmidt's results to be amenable to a polygenic interpretation, and *Drosophila* (Dobzhansky & Schultz, 1934) also appears to agree with the polygenic expectation.

Heterogeneous breeding systems depend on switch genes for their direct working, but are probably polygenic for the strength of control and general adaptation. The same conclusion has been reached by Ford (1937,* 1940*b*) for cases of polymorphism and it would appear to be a general principle, that even where sharp oligogenic variation is observable in an adaptive character, switch genes only provide the necessary trigger action, adaptation being secured by polygenes. In this way the development of such systems is gradual and their adaptation capable of continuous fine adjustment.

The adaptive changes in polygenic constitution may even bring about changes in the switch genes, new ones being substituted for old. This would appear to be the case with the system of sex determination in *Lebistes* and elsewhere (Darlington, 1934*b*). The whole system is capable of change and readaptation.

(3) *Breeding control and isolation*

The breeding system of a species, like any other phenotypical feature, cannot be immutable. It must be subject to change for the same reasons as are other characters, viz. that it represents an adaptation to existing conditions and must alter to meet new conditions if the species is to survive. Most species presumably do fail to survive because they fail to show adequate adaptation; but existing species, deriving from successful ancestors must afford some evidence of what is necessary for success in the breeding system, as in other characters. Furthermore, the evidence suggests that the breeding system changes by exactly the same means as other characters, viz. by selection acting on heritable variability of a polygenic nature.

The general principle that evolutionary changes can never be wholly or even largely reversed (see Muller, 1939) holds true with breeding systems. There is a steady progress

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towards more exact control, and even where the conditions favouring the mixed system characteristic of the early prototype are re-encountered after a controlled breeding system has developed, the successful organism will be that which shows new adaptation by the rise of a balanced inbreeding-outbreeding mechanism, not by a regression towards loss of control.

Such a change may occur either by adaptation of the existing method of control to the new conditions, or by the superimposition of a new type. Homostyly in heterostyled plants is probably an example of the former (Mather & de Winton, 1941). The outbreeding system is changed, in this case by a reshuffling of the switch gene components, to give an equally rigorously controlled inbreeding. It would seem that balanced systems can then be produced by mixtures of homo- and heterostyled individuals such as have been found in the wild (Crosby, 1940).

Examples of the superimposition of new mechanisms on old are many, and sometimes show evidence of complex series of changes. Wheat provides a simple case. The floral behaviour shows adaptation, and is often described as an example of such adaptation, to cross-breeding by wind pollination; but the anthers burst before extrusion, so giving regular self-pollination. Inbreeding is superimposed on outbreeding.

Some of the Compositae show a more complex situation. Arrangement of the flowers into a capitulum is most easily understood as an advantageous means of promoting outbreeding by attracting insects, and is usually accompanied and strengthened by protandry. But many species also achieve self-pollination by the device of curving the stigma lobes over until they pick up pollen from the anthers of the same flower. This was followed in *Taraxacum*, for example, by apomixis, which, by sacrificing the normal sexual process, secures even more rigorous 'inbreeding' and the immediate survival of cytologically anomalous types, though it equally determines the ultimate extinction of the species through prohibition of any extensive future change with selection (see Darlington, 1939). In *Dahlia*, on the other hand, a second cross-breeding mechanism, incompatibility, is in evidence (Lawrence, 1931), so marking a new break towards outbreeding. These complex breeding systems, inexplicable in any other way, are thus seen to represent stratified adaptations towards outbreeding and inbreeding, presumably recording the changing circumstances through which the prototype species passed successfully.

A remarkable feature of these stratified systems is that outbreeding is not often observed to have been superimposed on inbreeding, though the reverse is very common. The reasons for this are to be found in the consequences of inbreeding, which, as we have seen, increases fitness at the expense of flexibility. Inbreeding reduces variability, and an inbreeding species will thus be less likely to develop a new system than will an outbreeding species (Huxley, 1942). Furthermore, a species showing rigorous inbreeding is very likely to be exterminated sooner or later, because of this inability to change with condition, except for the very slight effect of mutation. An outbreeder, on the other hand, is pre-eminently flexible, can change with conditions and so can leave some successful descendants. Inbreeding must frequently lead to extinction while outbreeding need not.

The change from out- to inbreeding has far-reaching consequences. An outbreeding species will be capable of great variation by virtue of its store of variability, and so will be able to occupy and colonize a wide range of environments. This it must achieve at the expense of a decrease in present fitness, because high local adaptation will not be

possible. So long as competition is not so severe as to put an increased premium on high fitness, the outbreeding system, and hence wide adaptability, will persist. But if a higher standard of fitness becomes favoured, by increased competition, a change towards inbreeding will set in. The species or superpopulation will break up into small locally adapted populations which seldom interbreed. The polygenic combinations will become balanced within and not between these populations. Heterosis will occur when individuals from different populations happen to intercross, and the inbreeding system will tend to be strengthened and developed into an isolation mechanism (§ V (2)). Isolation and speciation will be the inevitable consequences of change from outbreeding to rigorous inbreeding (Fig. 9), though, if brought about in a different way, isolation may equally cause change of the breeding system.

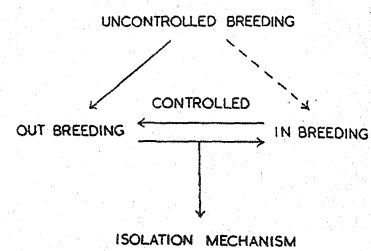

Fig. 9. Breeding control and isolation. There is a steady tendency to develop more rigorously controlled outbreeding from uncontrolled breeding. No evidence exists of a similar rise of inbreeding control, but this can be derived from, and may also give rise to, controlled outbreeding. The change from outbreeding to inbreeding is accompanied by the rise of isolation mechanisms (see text).

A further consequence will, of course, be that any of the new species which do not retain some measure of outbreeding, or the ability to revert to such a system, are doomed to extinction. To express the series of changes in terms of the variability states, we can say that outbreeding encourages the replenishment of free variability from the potential store, but that this power is lost when close inbreeding supervenes. The free variability immediately available permits high local adaptation, the potential variability being frozen by the inbreeding mechanism. Since future adaptation depends on release of this store, freezing, unless, as would seem rarely to be the case, reversible, means extinction, and supplantation by more flexible competitors.

Something of this kind has happened and is probably happening now in such groups of the genus *Drosophila* as that which includes *D. miranda* and *D. pseudo-obscura* (Dobzhansky, 1935). The former species has two partly isolated, and the latter two almost fully isolated, races. This implies developing speciation. It is accompanied by inbreeding, for Dobzhansky & Wright (1941) have evidence that *D. pseudo-obscura* consists of a large number of small populations showing free intra- and restricted interbreeding. Sexual selection also seems to occur (§ VI (1)). Presumably then, there has been a large freely outbreeding species. Increasing severity of competition favoured the rise of inbreeding which gave rise to speciation. The various species and, more especially, the two races of *D. pseudo-obscura* seem to be the relicts of a previous break up, having become isolated and so able successfully to populate common localities (§ V (2)). The strains within each race are evidence of a new break up now in progress (also see Huxley, 1939). It follows on our argument that many of the species recently developed or now developing are nearing the end of their history.

Wright (1940) and Dobzhansky (1941) have developed a different view of the part played by small populations. They point out that in such cases the random fixation of genes, or genetic drift, will cause the free variation to exist mainly between, rather than within, the populations. Since populations are not in direct competition this should preserve variability, since it will be less likely to be eliminated by response to selection. This reasoning is quite sound, so far as it goes, but two considerations, dependent on

polygenic behaviour, have been overlooked. These considerably lessen the importance of this particular consequence of small populations.

In the first place, the greater the number of genes affecting any character, the less is the importance of random fluctuation in the allelomorph frequency of a single gene. With polygenic behaviour each of the populations will settle down to a characteristic phenotypic distribution, broadly similar for them all, though dependent on different genes to an extent determined by population size (§ V (2)). The genotypic flux will not be represented fully in the phenotype, on which selection acts.

Secondly, Wright & Dobzhansky discuss only the free variability, though, as we have seen, there is reason to believe that polygenic variability is more potential than free. Reduction in population size will protect the free variability from selective diminution, but it will also decrease heterozygosity and so diminish the chance of freeing the potential variability. The quantity of free variability preserved in this way will be more than offset by the loss of new free variability caused by the reduction in transformation of potential to free. Thus the net result will be a loss of, not as Wright and Dobzhansky conclude a gain in, flexibility. The degree of protection of variability from the action of selection conferred by small populations, will be completely overshadowed by the closing of the variability store.

No matter what the mating system may be the major part of the variability must be potential and hence does not have any phenotypic expression. It will not be subject to the action of selection. Thus the value of small populations as a means of protecting and preserving variability will be insignificant, while their action in sacrificing flexibility to fitness, in increasing adaptation at the risk of extinction, will be of great moment. The latter action will determine population size.

VII. SUMMARY

The occurrence of natural selection demands (1) that there exists genetical heterogeneity, and (2) that unlike genotypes leave different average numbers of progeny. It is now known that both of these conditions are fulfilled, and all the available facts of evolution are in accord with the genetical theory of variation and selection.

Species must, on this view, differ in the same way as, but to a greater extent than, varieties or individuals of the same species.

The application of this criterion leads us to the conclusion that species differences are polygenic, i.e. depend on quantitative characters whose variation is controlled by many genes. These genes have individual effects which are both similar to one another and small when compared with non-heritable fluctuation. Other kinds of heritable difference are ancillary to polygenic variation in speciation.

Each individual polygene is inherited in the same way as the familiar major mutants of the laboratory. As, however, there are many polygenes affecting a given character, the aggregate type of inheritance is distinct from that of the major mutants. Polygenically controlled differences are quantitative rather than qualitative and do not lead to the sharp segregation shown by the more familiar genetical differences. Polygenic characters, such as stature in man, can show any degree of expression between wide limits. Many genotypes may have the same phenotype. Thus polygenic theory relates continuous phenotypical variation to discontinuous genotypical variation, the biometrical to the genetical.

These special properties of polygenic behaviour lead us to a new and clearer understanding of the action of natural selection in producing adaptive and evolutionary changes.

Very fine adaptation of the phenotype to environment is made possible by the existence of such a wide range of phenotypic expression. The frequency distribution of the individual phenotypes found in a population may approximate to a normal curve. It may, however, also be skew, to an extent determined by the dominance and interaction relations of the polygenes and by the scale on which the character is measured. The central, most frequent, phenotype must closely approximate to the optimum for the prevailing environment. Departure from this central type will thus mean poorer adaptation and loss of fitness.

The phenotype is produced by the genotype acting as a whole. Since polygenes have effects similar to one another, a given phenotype may correspond to various genotypes some containing one and some another allelomorph of a given polygene. As a consequence neither allelomorph will have an unconditional advantage over the other, in the way that major mutants do. Rather the advantage of any allelomorph of a polygene will be conditioned by the other polygenes present. Fisher's theory of dominance then leads us to expect that, in wild populations, equal numbers of polygenes will show dominance of the allelomorphs leading to increased and decreased expression of the character. Artificial selection disturbs this equality. The existing evidence is in keeping with these expectations.

The existence of polygenic variation free in the phenotype must lead to some individuals departing from the optimum and so showing reduced fitness. Variation is to this extent disadvantageous, but it is also essential for prospective adaptive and evolutionary change. The polygenic variability necessary for prospective change need not, however, exist as free phenotypic variation which will affect fitness. It may be hidden in the genotype under the cloak of phenotypic constancy, when it will have no effect in lowering fitness. Such hidden, or potential, variability is released, and shown freely by the phenotype, as a result of segregation from heterozygotes. Free variability may pass into the potential state by means of crossing between unlike individuals. Some potential variability will exist as differences between homozygous individuals. Such homozygotic variability can be freed by segregation only after intercrossing has rendered it heterozygotic.

If most of the variability in a population is potential, high current fitness can be combined with the possibility of great, though slow, change under selection. In such cases the response of the organism to selection will largely depend on the fixation of variability as it passes from the undetectable potential to the detectable free state. Thus selection may superficially appear to create its own free directional variability.

The frequency of recombination between polygenes affecting a character will control the rate of variability release. Consequently the effective recombination frequency is itself an adaptive character and will be subject to selective action. The evolution of genetic systems is largely the history of this selective control of effective recombination.

Control of recombination is almost wholly achieved within chromosomes, so that the storage of variability must depend on intrachromosome adjustment. Natural selection will tend to build up balanced combinations of polygenes within each of the chromosomes. These combinations will have the properties of close adaptation to the optimum, great variability storage and slow variability release.

Combinations are characterized by two kinds of balance, that of the individual com-

bination, as shown in homozygotes (internal balance), and that of pairs of combinations when working together in heterozygotes (relational balance). Dominance permits the adjustment of these balances independently of one another. The theory of polygenic balance shows how polymorphism and clines can be maintained.

Heterosis is due to a particular kind of poor relational balance brought about by artificial selection. The concept of heterosis is now extended to include all types of such unbalance, natural and artificial. Poor relational balance encourages isolation, and so heterosis, in this broad sense, stimulates the rise of isolation mechanisms and hybrid sterility.

The store of polygenic variability, steadily depleted by random fluctuations in allelomorph frequency and by response to selection, is replenished by new mutations. Since all polygenes affecting a given character have much the same effect, the phenotypical properties of a population may be stable or nearly so even though the genotype is fluid. Fixation, mutation, segregation and recombination cause a genotypic flux to exist under the cloak of a phenotypic stability, itself maintained by the action of the same natural selection, which, under new conditions, would lead to new adaptation.

The mechanical relations of unlike combinations, whose constituent polygenes are intermingled along the same chromosome, are sufficient to account for the degeneration of unused organs.

The breeding, or mating, system of a species determines the frequency of heterozygosity, upon which the rate of release of potential variability depends. Inbreeding gives homozygosity and high immediate fitness; but it freezes potential variability in the homozygotic state and so reduces the chance of prospective adaptation. Outbreeding has the reverse effect and sacrifices some fitness to flexibility.

The breeding system is thus an adaptive character. It will be subject to selective change towards more closely controlled inbreeding or outbreeding. A controlled compromise between inbreeding and outbreeding may also occur. The strength and direction of control is probably polygenically determined, though the actual controlling mechanism may depend upon a major switch gene for its direct action.

A change from outbreeding to inbreeding increases local adaptation and so leads to heterosis and isolation. It also freezes potential variability and lowers the chance of prospective adaptation. Thus a species which shows such a change to inbreeding will break up into a swarm of small, locally fit, but inflexible, new species. As a consequence of their inflexibility, most of these must perish when environmental changes set in.

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THE DYNAMIC APPROACH TO PLANT STRUCTURE AND ITS RELATION TO MODERN TAXONOMIC BOTANY

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I. HISTORICAL INTRODUCTION

Plant structure has been viewed from widely different standpoints in the course of the history of botanical science. From a mystical approach whereby the location of the plant's soul was considered profitable matter for discussion, has emerged an attitude which regards the vegetable body as a mechanism, albeit infinitely complex in its workings. Similarly, as scientific enlightenment has increased, descriptive botany has come to be set against a new background. If it be true that a perfectly static view of any living organism would have been impossible even in the earliest times, it is an equally valid claim that the attainment of a truly dynamic approach to plant structure has been the development of the past century.

The pioneer investigators Malpighi (1675) and Grew (1671) studied structure with some regard for the functions of organs described; but their ideas on plant physiology consisted almost entirely of conjecture. Hales (1731) introduced a more scientific mode of approach, but his hypothesis of 'sulphur, light and aer' combining to 'invigorate the seminal plant' is sufficient evidence of the trend of current speculations concerning life histories. Each generation was clearly a distinct entity, miraculously formed, in the judgement of these early workers.

In the realm of taxonomy John Ray attempted a 'natural' classification in 1686, but it appears that his sole concern was the association together of those plants which he considered to have been created with obvious similarities. Linné, when he published the famous aphorism 'Natura non facit saltus' (1751), was equally far from a conception of evolutionary progress. Linné was no more satisfied with the 'sexual system' of classification which he devised than were Jussieu (1789) and A. P. de Candolle (1813). But even to these men—foremost systematists of their day—the species was something established 'in the beginning', and immutable, save for the occurrence of a limited number of 'mule-plants' or hybrids.

Thus in their conception alike of the individual generation and of the separately created species the earlier botanists contrived to work with stabilized units in the formulation of their systems. Furthermore, in their descriptions they attributed to each part of the plant body an organic individuality out of all proportion to the observable facts. Clarity of definition was the hall-mark of good descriptive work. Just as there were distinct 'species of vegetable', so there were in any one specimen several 'species of organ'. This attitude was tenable in the absence of any evolutionary theory. Moreover, it was exceedingly convenient.

As an instance of orthodox botanical thought in the opening years of the nineteenth century one may cite the remarks of an American professor of botany, Dr Barton (1803), on the subject of cross-pollination: in a brusque dismissal of the question, he condemned as 'feeble and visionary' the 'theory of those philosophers who have imagined that Nature has connected together, in necessary dependence, her innumerable productions, like links in a chain of man's construction'. This weird opinion was voiced long before the birth of the modern science of ecology. But it is well to remember that in some such mental atmosphere the foundations of present-day taxonomy were laid. More indeed than the foundations of taxonomy were laid in those times. Pursuing the metaphor, one might say that the very walls of the modern taxonomic house have been fashioned with Linnaean bricks. And this would be no idle fancy. Rather is it of the highest significance that the units genus, species, and variety, in addition to the larger categories—class and natural order, and a host of terms such as ovary, style and stigma, were devised and established considerably before the dawn of a dynamic approach to any branch of structural botany. Small wonder, therefore, that conflict should exist between taxonomy and the great branches of botany that have appeared so much later upon the scene. The nature of this conflict, here to be considered, constitutes a study germane to a number of the more important botanical controversies of our time.

Just as the early botanists constructed a so-called natural classification before they challenged the doctrine of the immutability of species, so the first hint of a concept of homologies, curiously enough, far antedated the acceptance of the theory of descent. The explanation lies doubtless in the fact that Goethe's 'Doctrine of Metamorphosis' (1790) was essentially metaphysical, whereas the establishment of homologies in the modern sense must of necessity rest securely on biological observations. It is less easy, however, to dismiss so cursorily the work of Wolff (1759), who arrived at his conclusion that 'all

parts of the plant except the stem are modified leaves' as a result of studies of development. These men propounded the idea that organs of widely different appearance were referable to a few fundamental types. But they failed to take a step further in the direction of dynamic thought and thus provide the only explanation of how this could be so.

It is evident that a considerable element of confusion prevailed in botanical thought during the first few decades of the nineteenth century. A fair body of descriptive matter had been compiled, but referring to the standard text-books of the time one finds that buds were classed apart from stem or leaf, under the quaint head 'hybernacula'; while stipules continued to be grouped with other entirely different structures among the 'fulcra', or props. Tissues had been studied and vessels described with tolerable accuracy, but it remained for Robert Brown (1866) to demonstrate the existence of a nucleus, and for Schleiden (1849), von Mohl (1852) and Naegeli (1858) to commence the elucidation of the living substance in plant cells. Philosophers, more than working biologists, had dallied with the notion of 'metamorphosis' of plant organs. A few penetrating thinkers had begun to question the truth of 'special creation'. Meanwhile the orthodox systematist was engrossed in the description of herbarium specimens in a manner which showed him to be a true disciple of Linné. It was in this atmosphere that Charles Darwin received his early biological instruction. The science of structural botany has changed profoundly since that time; certain practices of taxonomy but little.

Among the less orthodox biologists of the day, release had been sought from the stultifying concept of specially created species before either Darwin or Wallace had embarked on a scientific career. The views propounded by Lamarck (1809) are well known, but it is sometimes forgotten that an observer named Matthew put forward views in the year 1831 which, according to Darwin himself, showed that 'he clearly saw the full force of the principle of natural selection'. When von Mohl, Naegeli and others had elucidated with such minute care the finer structure of plants, the stage was indeed set for the appearance of two publications that were destined to shake the very foundations of vegetable morphology. These were the researches of Hofmeister (1849–56) collected in his *Vergleichende Untersuchungen höherer Kryptogamen*, and Darwin's *The Origin of Species* (1859). If Darwin is so much more widely known than Hofmeister, that is largely the result of the human implications of the zoological side of his work. From the botanical standpoint it is clear that each unconsciously served the purpose of the other. For while Darwin propounded the first reasonable theory by which species could be viewed as part of a continuous organic development, Hofmeister furnished the morphological details and pointed out the existence of homologies which alone could lend credence to Darwin's doctrine in its application to the plant kingdom as a whole. A dynamic approach to structural botany was in sight.

The remaining forty years of the century saw an unprecedented output of detailed morphological work, in large measure the direct outcome of a natural urge to fill in the details of a map whose broad features Darwin and Hofmeister had so temptingly sketched. As Harvey-Gibson (1919) has said, a notable feature of the period lies in the extent to which the different branches of the science were drawn together. To the cryptogams, formerly considered as a group apart, the student of the higher plants would turn in his quest for origins. Every year new facts poured in, always to be filtered through the sieve of evolutionary thought. A fluidity of outlook seemed assured.

Here, however, one meets with a curious anomaly. For while the boon of an emancipated viewpoint had been provided, the maturity of scientific thought necessary to make full use of this boon had not yet appeared. So it came about that, working on the initial assumption of evolution by the slow operation of natural selection on slight, random variations, botanists were induced to formulate morphological canons differing all too little in essence from the early metaphysical assertions of Goethe and his contemporaries. One may cite, for example, the stelar theory of van Tieghem (1886). This theory, however interesting as an aid to phylogenetic discussion, leads too readily to the dangerous implication that the stele possesses a structural individuality apart from the surrounding tissues. Similarly, the carpel theory, which Schleiden (1849) had derided, seemed doubly attractive in the light of the generalizations of Hofmeister, and acquired a new significance in its application to the problems of evolution.

The forty years which saw the production of the classical text-books of Sachs (1875), de Bary (1884) and Strasburger (1898), were pre-eminently a period of the accumulation of facts subordinate to a consistent theme. Current work on the adaptational aspects of flower structure culminated in Knuth's monumental *Blutenbiologie* (1898 to 1905). Meanwhile Solereder (1898) was engaged on his *Systematische Anatomie*, and Coulter & Chamberlain (1901) in America were preparing a comprehensive account of phanerogamic embryology. In all these different branches of work the one common aim was the ultimate completion of the evolutionary picture.

One must beware, however, of too strong an insistence on any homogeneity of approach to morphology among the botanists of that period. The trail that T. H. Huxley blazed in the zoological field finds no complete counterpart in the realm of botany. Evolutionary evidence drawn from ontogeny, which took an important place in Darwin's original argument, was drawn principally from the animal kingdom. No great certainty prevailed on the matter of what structures in plants would permit of recognition

as distinct entities, and of consequent clarity of definition. Divergence of opinion was quick to show itself, and important classical tenets had been challenged before the turn of the century. Thus we find that in 1901 Bayley-Balfour wrote of the monocotyledonous cotyledon that it was 'not a leaf nor the homologue of the lateral cotyledons in Dicotyledons'. Again, Coulter & Chamberlain complained in 1901 of the hard categories and rigid morphological concepts of what they styled the 'old morphologists'.

The year 1900 saw the rediscovery of the all-important writings of Gregor Mendel (1866), at a time when the work of De Vries (1901), Bateson (1894) and others was beginning to attract attention to a new field of biological science. Facts of crucial significance which had remained buried for thirty-four years were at last brought to light. And these facts were not only to provide, under the title of 'Mendelism', the basis of modern genetics, but they were to exert a potent influence on almost every department of biological inquiry. The power of genetics over morphology lies not so much in the factual content of the former branch of biology as in the mode of thought which it engenders. With the birth of modern genetics, and its growth by way of experimental and cytological studies, a new flood of light has come to be thrown on the significance of structural characters. At the present time we tend, perhaps, to be dazzled by the new light rather than able to see structure more clearly with its aid. But one point is clear. The last vestiges of justification for a static view of plant structure have vanished.

This short historical sketch draws near the point of the present day: and it meets with certain characteristic features of the modern approach to plant structure. The position is, however, somewhat obscured by two circumstances. One is the immense output of work within the past forty years which might be claimed to have some bearing on the present question. The other lies in a curious paradox observable in modern biology as a whole; the paradox that, with ever-increasing specialization of workers into narrow fields of intensified investigation, there has at the same time been a growing tendency towards interdependence between the various main branches of the subject. If it be true that the modern views on plant structure will permit of no very clear-cut analysis, it is equally true that certain general features can be detected as characteristic of the outlook at the present time. One of these features is a fluidity of concept, the direct outcome of a dynamic approach which is evident in a number of distinct fields of work. It is perhaps no accident that the same year—1908—saw the publication of Bower's *Origin of a Land Flora*, Lignier's *Essay on Morphological Evolution*, and Tansley's *Notes on the Filicinean Vascular System*; for at that same period doubts about 'Darwinism' were fast growing, while data concerning heredity were accumulating apace. Here were no isolated pieces of research, but rather expressions of a trend; and this trend is essentially in the direction of a completely dynamic approach to plant structure.

It would be beyond the scope of the present article to discuss more than a few of the outstanding aspects of a trend so wide in scope. Accordingly, this study will be limited to the morphological and anatomical fields, in which there are distinct indications of a changing point of view. Following the presentation of certain evidence drawn from these fields, it will be possible to consider briefly the new approach in its special relation to taxonomic problems.

II. EVIDENCE OF A DYNAMIC APPROACH TO PLANT STRUCTURE IN MODERN MORPHOLOGICAL AND ANATOMICAL INVESTIGATIONS

Few publications in the domain of structural botany within recent years have given rise to more bitter controversy than those purporting to establish a healthier point of view in matters concerning the flower, and, in particular, the gynaeceum of angiosperms. In the originality of their contentions such observers as Grégoire and McLean Thompson have struck at the very heart of morphological theory. These men have propounded views based largely on the facts of ontogeny, and for thus limiting the source whence their conclusions have been drawn they have met with considerable criticism. It is probable, however, that the opposition with which these views have met hitherto is in large part attributable to a second underlying cause, the nature of which will become clearer at a later stage in the present discussion. It will be convenient here to consider briefly some of the main contentions embodied in their work.

The essence of McLean Thompson's contribution (1934) undoubtedly lies in his refusal to accept the ovule-bearing carpel as a fundamental constituent of all complete angiosperm flowers. Nobody who had watched the distinct mode of origin of styles and placentae in the typical scitaminean flower, or who had traced the origin of some of the carpels of an apocarpous flower to one, others to the union of three separate primordia, could fail to entertain doubts concerning the existence in fact of the carpels so highly prized in theory.

Grégoire's outstanding researches (1931) on the gynaeceum led to conclusions in agreement with those of McLean Thompson in their denial of the existence in flowering plants of foliar ovule-bearing organs. McLean Thompson stands out, however, in his insistence on carrying an emancipated viewpoint to its logical conclusion of almost complete morphological agnosticism. 'Stigmatism', he writes (1934), 'is a state of tissue. There is no such organ as a stigma, comparable with other organs such as stamens and petals.' Elsewhere, he proclaims (1937),

'We are indeed at a new beginning. Contrary to the teaching of the past, we know that Angiospermy is not yet understood.' It is clear that, as he has himself expressed it (1937), we have here a 'portal' rather than a clear forward path. If we pause to inquire of the morphologist concerning the way ahead, he tells us (1934) that 'the real problem of floral morphology is that of the physiology of growth of a sporogenous axis'. To some this statement involves an intolerable contradiction, but as we examine further instances of a new approach to plant structure we see it in its true significance, as the unavoidable end-point of one of the most characteristic trends of modern botany.

Turning to examine some of the more recent work on apices in general, one finds that a stimulating review paper (Foster (1939)) has recently been published. In this concise summary the reviewer contrasts the 'tunica-corpus' theory of Buder (1928) with the old and more rigid alternative theories of Hofmeister & Naegeli (1878) (apical cell) on the one hand, and of Hanstein (1868) (histogen) on the other. Some apprehension is expressed lest the tunica-corpus theory should share the fate of its predecessors, in that it too might in time fall a victim to attempts at rigidity of interpretation. For the present, Foster writes of this theory, with evident satisfaction, that it 'serves to focus our attention on the dynamic rather than the purely formal aspects of the shoot apex'. Singular interest attaches to Grégoire's observations (1938) which led him to distinguish in the floral apex a distinct 'manchon méristematique', and his contention that procambial strands in the inflorescence develop acropetally may indeed, as Foster asserts, constitute a 'unique and fundamental contribution'. But it is a contribution that could readily lead on to a new formalistic dogma. Even in matters botanical few can tread for long the 'tightrope of agnosticism'.

At the present time no new wall of dogma has been erected around current work upon the apex in the shoot of higher plants. Rather is it evident from recent publications that a changed and less rigid attitude is being assumed also towards the various tissues arising from the meristematic region. This is indeed as might be expected; and the stimulating work of Louis (1935) and Esau (1938–9), among others, forms a natural corollary to an emancipation from the formalism of Hanstein in the interpretation of the apex.

The most notable feature of Esau's comprehensive studies of the ontogeny of phloem tissue is the extent to which old and long-accepted dicta are found to be inaccurate or otherwise unsatisfactory interpretations of the observed phenomena. A single example may be cited by way of illustration. Since the days of van Tieghem, the fibres which occur in a position immediately external to the phloem in the herbaceous stem of *Nicotiana* have been classed as pericyclic fibres. But a careful study of the developing tissues, in the absence of preconceived opinions concerning homologies, has convinced Esau (1938) that the ready separation of 'pericyclic' fibres from adjacent phloem tissue has no foundation in fact. A most refreshing conception of phloem tissue is engendered by the reading of Esau's critical accounts, so different is it from the formalized picture with which all are familiar in botanical text-books. It is a dynamic view whereby this tissue is seen as an aggregate of living elements in all the complexity and variety of their developmental phases, inextricably associated with all other elements adjacent to them in time and space. This view permeates all her judgements. It is seen in the considered opinion voiced on the relative status of cambium and procambium. It is evident in her remarks respecting the gradual transition from metaphloem to secondary phloem. Throughout, one is conscious that processes rather than structures are the ultimate objects of description.

There is an obvious parallel between the trend of work just considered and the recent contributions to floral morphology discussed above. In both cases we find insistence on the functional aspect of the structures observed; in both there is a tendency to expose long-established beliefs as fallacious; and observers in both these fields can silence critics of the 'ontogenetic mode' with the declaration that development, in the fullest sense, is co-extensive with life itself. One difference between the work of Esau on the ontogeny of phloem and that of McLean Thompson on the ontogeny of flowers lies in the fact that, whereas the former professes to be studying anatomy, the latter emphasizes the morphological issues involved. In the one case dogmas are tacitly removed; in the other they are vigorously assailed. Few would raise a murmur at the disappearance of the pericycle as a structural entity in the herbaceous stem; but many voices join in defence of the carpel as an integral component of every perfect angiosperm flower.

A trend which is apparent in more than one line of investigation of the primary body may be expected to appear in like manner where consideration is given to the structure of the secondary tissues in higher plants. From the formalized descriptive work of the late nineteenth-century anatomists, to the 'canons of comparative anatomy' propounded by Jeffrey (1917), was a natural step. But the formalized interpretation of the structure of the secondary body enunciated by Jeffrey arrived upon the scene too long after its counterpart in floral morphology to meet with an altogether kindly reception. Among those who rose in opposition to these views, and who approached the same problems from a more modern angle, were Bailey (1920, etc.) and his co-workers, Sinnott (1914), Frost (1931), Kribs (1935) and others. In a long series of papers on 'The cambium and its derivative tissues', Bailey (1920, 1923) led the way towards a view of secondary tissues which would be emancipated from any preconceived dogma and

would at the same time rest upon a secure foundation of accurate and detailed observation. Constantly alive to physiological implications, and invariably considering a tissue in its developmental entirety, Bailey provides an illustration of a viewpoint that is essentially dynamic. His critical studies of cambial initials are paralleled almost exactly by the still more recent work of others on procambial differentiation. The peculiar value of both lies in the emphasis placed on pure observation.

The instances here offered in illustration of a particular approach to plant structure might readily be multiplied. Salisbury (1931), for example, has published a stimulating account of *Ranunculus parviflorus*, where he examines structure in all its variety of significance. Again, the name of Priestley (1928) is associated with much suggestive research, although so pronouncedly physiological as to fall somewhat outside the scope of the present discussion. Thoday (1922), also mainly a physiologist, has assisted materially in clarifying our conception of structure in so familiar a plant as the common sunflower (*Helianthus*). Like Salisbury, he brings long experience in the study of physiological anatomy to the task of resolving wider problems; and like many others he is refreshingly modern in his manner of attack on these problems.

Enough has been offered by way of illustration from some of the fields in which microscopical anatomy plays a leading role. Not all those quoted above as evincing in some measure the new approach would be in sympathy with one another's opinions on particular issues. But few of these botanists would find themselves at variance with Schuepp's complaint (1936) that 'our comparative morphology deals always with adult forms without proper regard to their method of origin'. And it is probable that all would willingly extend Foster's remarks concerning the shoot apex to the plant as a whole, agreeing that 'what is urgently needed in advance of any theory of phylogeny is a broad and sound knowledge of structure in a wide series of both Gymnosperms and Angiosperms'.

It is altogether natural that, while a new outlook on anatomical structure has been spreading, a like trend of thought should have become apparent in the more strictly theoretical concepts of gross morphology. As in histogenetic research, so in morphological debate, a series of recent publications has embodied ideas foreign to the formalized approach characteristic of an earlier period. Indeed, no sharp line can be drawn between these different manifestations of a single modern trend. Among those who, in their writings on the morphology of the vegetative body, break away from old traditions, Arber (1925) is a conspicuous example. Her extensive studies of plant organs, particularly a series of detailed observations on members of the Gramineae (1934), have led to some remarkable conclusions concerning the validity of hitherto generally accepted morphological categories. Instances are cited in which, one by one, the characters serving to distinguish stems from leaves break down. Faced with the terminal foliar organ of certain bambusean spikelets and other parallel examples of organographic irregularities, Arber seeks refuge beneath the shelter of the cauloid theory of Lignier. Explaining her point of view, she writes (1930) that 'if we once accept the fact that stem and leaf are no more than convenient descriptive terms, which should not be placed in antithesis as if they corresponded to sharply opposed morphological categories, the problems of their delimitation and of their differentiating characters vanish into thin air'. So too does a great part of interpretative morphological science.

To Thomas (1932), who is also a revolutionary in this respect, the disappearance into thin air of the 'old' morphology would be entirely desirable. For in his eyes the complete system embodied in the leading morphological works of a generation ago represents 'a great edifice built on sand which crumbles away whenever it is closely investigated'. He is insistent in his demand for an objective approach in place of the subjective outlook which, he claims, has prevailed in the past. Out of his long experience in the critical examination of fossil floras Thomas brings the suggestion that the difference between megaphyll and microphyll may be as great as that between stem and root. Out of a life study of living plants emerges Arber's conclusion that leaf and stem are no more than convenient descriptive terms. Opinions of such weight, arising from two distinct lines of evidence, cannot pass unheeded.

In the hands of those who advocate a new approach to these matters it is clear that the old morphological categories—wide or narrow, external or histological—continue to exist merely as tools of description. Indispensable but undesirable, when not actively deprecated they are tacitly ignored. For increasing numbers of morphologists and anatomists are no longer prepared to consider structure in terms of static concepts. Some observers, it is true, would merely replace one troublesome theory by another that might prove in practice to exert a still more restricting influence. The theory of 'Carpel Polymorphism' (Saunders, 1925), for instance, is open to this charge (Eames, 1931), and it is doubtful whether Saunders, by proposing her 'Leaf-skin' theory (1922), has done more than consign an old morphological problem to a new histological domain.

Accumulating evidence respecting the floras of remote antiquity has insured a long-term view of the organs represented in existing plants. The germ of the telome theory lay long in the fossils of Devonian rocks, before the brilliant researches of Kidston & Lang (1917–21), among others, revealed the detailed structure of vascular plants more primitive than any that had been previously known. Meanwhile a changed attitude towards the structure of the living plants had become apparent on many

sides. Organs and tissues were seen in the scale of geological time. They had also come to be seen in the time scale of the individual life. In each case the widened view appears to have engendered an abhorrence of all hard categories. Still another time scale was slowly coming to be appreciated—the essential link between the other two, the time scale of the geneticist. How far the rise of the experimental study of heredity has contributed towards the growth of the particular dynamic trends indicated above is not clear. It is in a somewhat different capacity that this study has exerted its most important influence on structural concepts. Not the individual, not the race viewed in terms of geological time, but the species—or short-term racial unit—has been the weak point assailed directly and indirectly through genetical inquiry. This involves questions more appropriately discussed at a later point, in the context of taxonomy.

The dynamic approach to plant structure is thus seen, on examination, to be reflected in a wide range of modern publications concerned with the morphology and anatomy of the higher plants. That it has a very real existence is evident when one raises the question, 'What has become of the old morphology?' For the controversy of sharply divergent views rages on all sides. Studies strictly in accord with the classical concepts continue to pour forth from the laboratories of Eames and others, while within the past two decades more than one authoritative textbook of morphology has appeared without any noticeable infusion of the modern fluidity of concept in its hundreds of pages of close descriptive matter. To both Goebel (1933) and Troll (1935) the 'Gestalt' is of the highest importance, and to extend morphology far beyond the systematic comparison of structural 'type' is to overstep the bounds of the subject. If morphology had not signified one thing to Grégoire, another to Goebel, the author of the *Organographie* would not have written of the great floral morphologist, as he did in 1933, 'dasz man ein ausgezeichneter Cytologe oder Anatom sein und doch der Morphologie gänzlich fremd gegenüberstehen kann'. Velenovsky (1907) must have been employing the term in a similarly restricted sense when he claimed that ontogeny had no place in morphological enquiry. A new attitude has made its appearance; but it is far from being universally established in the botanical world of to-day.

The concept of phylogeny was the outstanding contribution of the nineteenth century to the progress of a theory of plant and animal structure. Although Zimmermann (1930) has recently claimed a separate existence for the science of phylogeny, it represents nevertheless the meeting ground of taxonomy and structural theory. Accordingly, it may not be inappropriate to conclude the present section of this study by quoting a statement recently given by Thoday (1939), epitomizing the modern dynamic approach towards the central problem of the origin and development of the floras of the world. 'Natural selection of harmonious changes', he points out, 'may have been more important than elimination of functionally unfit mature organisms. It is developmental harmony which is important, of which functional efficiency of adult organs is but a part'; finally, 'if we can obtain light on the laws of harmonious development we may be in a better position to understand the nature of those major changes which the pageant of evolution unfolds'.

III. DISCUSSION, WITH SPECIAL REFERENCE TO THE TAXONOMY OF THE ANGIOSPERMS

Taxonomy, the science of arrangement, appeared long in advance of the advent of modern biology. In a primitive form it found expression in the earliest groupings of plants by classical writers into trees, shrubs and herbs. In a form retained in large measure to the present day it took shape in the hands of the illustrious Linné, acknowledged master of systematic method. That department of botany which is generally understood to-day by the term taxonomy is, however, considerably more comprehensive than anything which came within the orbit of the systematist of 1750. Nevertheless, the underlying purpose of the subject remains unaltered, in that its primary function is that of systematic arrangement, so that we are justified in regarding a fair portion of the enlarged 'systematics' of to-day as something that has come gradually to be superimposed upon the original taxonomic foundation.

In the appearance within the last few decades of a series of phylogenetic systems we see evidence of a superstructure of the kind indicated above. It is true that Small (1917–19) has considered Cassini (1826, 1834) as the author of the most generally acceptable scheme of relationships within the Compositae, and it must not be forgotten that all the more enlightened taxonomists of the early part of the nineteenth century were fast awakening to some conception of affinities. But a historical examination of the question has none the less clearly indicated that it was only with the acceptance of Darwin's evolutionary doctrine that a natural classification came, in the modern sense, to preoccupy biologists and to exert an influence on the different systems propounded by taxonomists. Thus, it is well known that Bentham (1863) long remained a believer in 'special creation'—if by such an ambiguous expression is implied the idea of constancy of species; and it is doubtful whether Engler & Prantl (1889–91), in their *Pflanzenfamilien*, intended more than an approximation to a natural classification. Only gradually did the 'phylogenetic tree' make its entrance and achieve its ultimate popularity. Lam (1936) has traced, with interesting results, the general course taken, and the diversity of form attained by this widely used phylogenetic symbol, from the time

of its introduction by Haeckel (1866) to the evolution of its modern three-dimensional counterpart. The acceptance of the phylogenetic tree as a useful implement of the taxonomist led naturally to the assumption of the role of phylogenetic interpreter on the part of the more enterprising workers in the taxonomic field.

It is no surprise, therefore, to find within the last forty years a number of publications purporting to offer that classification, of either large or restricted groups of plants, which approximates most nearly to the 'true' or natural course taken by the group in question in its evolution. Of large-scale interpretations of the angiosperms, we have those of Hallier (1912), Wettstein (1901), Bessey (1915) and Hutchinson (1926, 1934), to mention those that have perhaps attracted the greatest attention; and within each restricted group—family or genus—each expert has usually been found ready to voice an opinion in conflict with that of another competent observer concerning the correct interpretation of the evidence. Since the diversity of the conclusions reached constitutes one of the most characteristic features of systematic work of this category, it is logical to inquire how far it is within the province of taxonomy to offer interpretations of this kind, and exactly what is the nature of the evidence on which these divergent conclusions rest.

An answer to both these questions may be sought by reference to a somewhat earlier period in the history of botanical thought, in fact that brief spell during which an immense impetus was given to the study of structure as the sole available means of reading the obscure pages of the evolutionary book. It seemed obvious that organs could be divided readily into two classes, 'plastic' and 'conservative'. Finer structures could no less readily be grouped into 'Anpassungsmerkmale' and 'Organisationsmerkmale'. Through the agency of such a scheme it was a comparatively simple matter to decide on the extent of survival value or relative conservatism of any individual organ or structural peculiarity. Thus the taxonomist, studying structures, would find himself in a position to formulate schemes of descent. So it came about that systematists, ill-contented with their role as experts in the science of arrangement, took upon themselves the far more exacting task of revealing to the world the story of organic evolution. For the ideal 'natural system' could be none other than a plan of the course of evolution itself. Thus, adhering to the accepted principles, Wernham in 1912, and Sprague in 1925—to mention two typical instances—were able to offer plausible and intelligible sketches of portions of angiosperm evolution. One might multiply indefinitely these illustrations of the manner in which phylogenetic taxonomy has assumed the role of interpreter of structure in relation to evolution. But it is necessary only to glance at Hutchinson's recent volume on the monocotyledons (1934) to see in what manner the perianth may replace the endosperm as the 'fundamental criterion' employed by a systematist. The fickle nature of the 'evidence' is apparent.

The essence of one part of the modern conflict between the newer, more dynamic, approach to plant structure and the science of taxonomy lies indeed in this question: what exactly is the nature of the evidence on which conclusions may safely be drawn? It is here, for example, that the acceptance of McLean Thompson's 'Acarpous theory of modern flowering' would entail a mortal blow to existing theoretical morphology, by depriving it of value in the work of systematic interpretation. It is here, moreover, that we must look for an explanation of the great diversity of opinion on such controversial questions as the origin of the angiosperms and the early history of the monocotyledons. The familiar, but glib, citation of the Magnoliaceae as the most primitive type of living angiosperm can scarcely command the support of those anatomists equipped with a knowledge of some of the distinctly specialized finer structures occurring within the family.

At this point it will be convenient to examine certain of the repercussions of modern views upon the actual working units of the taxonomist. Dominating these views was the rapidly accumulating work on the mechanism of the evolutionary process. In the writings of Guppy (1907) and Willis (1907) can be found evidence of a profound mistrust of the Darwinian conception of the mechanism of evolution, and as a logical consequence, suspicion of interpretations based directly on that conception. Postulating 'immense syngamia' in place of Darwin's ancestral species, Willis and others have pointed to evolution in which isolation of genotypes must clearly have constituted a factor of very considerable importance. That 'heterozygotes will produce homozygotes but not the reverse' is a Mendelian contribution of fundamental importance to any such doctrine as this, a fact pointed out by Du Rietz (1930) in his admirable review of the whole subject of taxonomic units. It is unlikely, however, that all will share Du Rietz's conclusion that 'isolation has thus ousted natural selection as the dominant evolutionary factor', and thereby ignore the mathematical demonstrations of such experts as Wright (1930), Haldane (1924–32) and Fisher (1930). But even if such a view would appear to take insufficient account of mutation within the hypothetical isolated population, it is significant in coming from one with the authority of Du Rietz.

Equally significant, and considerably more spectacular, are the expressions of extreme iconoclasm that have emanated from more than one leading taxonomist within the past few decades. Lotsy's conclusion (1916) that 'phylogeny is but a product of fantastic speculation' is well known, but less generally familiar are the views to which the great Japanese systematist Hayata (1921) was drawn as a result of prolonged study of the taxonomy of higher

plants. Enunciating his 'participation theory' of evolutionary progression, Hayata writes thus: 'An innumerable number of organic beings have existed from the eternal past and will exist to the eternal future; they unite with and separate from one another and produce many different organisms by different combinations of the genes; or they change by themselves, as the genes change.' So clear is the parallel between this conclusion and the verdict of some modern anatomists that any biologist who accepted the principle of Hayata's theory would inevitably fall into agreement with Arber in expression of a complete agnosticism in almost all questions referring to the course of evolution. Those who would support Hayata must perforce admit, with Arber (1925), 'that the secret of the great underlying principle is entirely hidden from us', and that 'we have never yet succeeded in lifting so much as a corner of the veil which hides the mystery itself'—the mystery of progressive, organic evolution.

Taxonomy, it must be re-emphasized, is the science of arrangement, or systematic classification. As such it has had, and will always continue to have, a part of the first importance to play in the theatre of biology. The practising taxonomist is concerned in the first place with matters of description and nomenclature; only secondarily with relationships. When it is realized that the oriental species alone of the genus *Dioscorea*, for example, have increased through new discoveries from 45 to 154 since 1900 (Prain & Burkill, 1939), and that a very large proportion of tropical angiosperms are known only from the original collection whence they were described, it is possible to appreciate something of the task, in the form of description and nomenclature, which continues to confront the practising taxonomist. Every item of this great body of descriptive work must be carried out in precise and generally acceptable terms. Every term employed must refer to some distinct and recognizable structure or structural condition. Terminology comprises the very groundwork of taxonomy, a groundwork laid down, as we have observed, by the effort and organizing power of Linné nearly 200 years ago.

We are now in a position to view the central conflict between taxonomy and some of the modern work on the dynamic aspects of plant structure. That every system of classification since the time of Jussieu has been based in part at least on indications of natural relationships will be admitted. But to suggest that any organ, stigma or carpel, stem or leaf, is without a separate existence in reality and must henceforth be banished from phylogenetic discussion is to imply that the taxonomist who would have his descriptions scientifically true must construct those same descriptions without the essential tools employed by successive generations of his predecessors for more than 150 years. No description of a *species nova* includes an account of the pericycle. Hence the judgement passed by Esau on the formalism of van Tieghem causes no stir in the realm of pure taxonomy. But every account of a species is complete only when the gynaeceum has been considered. Consequently 'acarpous flowering' spells confusion to the taxonomist, trained in the use of the established tools of nomenclature. Further, when taxonomists of the experience of Willis, Lotsy and Hayata have voiced opinions in direct contradiction to the older, classical tenets of phylogeny, it is no longer possible to remain blind to the conflict here involved.

It appears, on reflexion, that the science of taxonomy is offered, as it were, a choice between two alternatives. Its most fundamental unit—the species—having been defined as no more than 'a momentary realisation of a line of evolution' (Faegri, 1935) and its entire terminology challenged, it can either undergo a revolution within itself, thereby falling into line with advancing thought, or it must cease to lay any claim to embodying a partial reconstruction of the course of evolution, and hence admit freely that the best of 'systems' can represent little more than an elaborated card-index, while the best of descriptions will be fashioned with terms biologically nearly meaningless and employed solely in the interests of convenience. In 1925 Sprague wrote: 'The taxonomist has a twofold task: to trace the general course of evolution, and to distinguish the numerous separate and often parallel lines of descent.' Scarcely a year later no less an authority than Coulter (1926) made the announcement that 'biologists are no longer concerned with the whole story of evolution, but only in discovering experimentally how one species may produce another one'. It would be difficult to find two statements, both coming from botanists versed in the taxonomic implications of plant structure, illustrating more characteristically the conflict between the old complacency and the new critical approach. Certain it is that taxonomy is without the machinery for fulfilling the extravagant claims made for it by Sprague in the context cited; but surely the ultimate goal of biologists in their critical study of heredity is a clearer view of the 'whole story' of evolution.

The crux of the problem here at issue lies in the precise role that one is prepared to assign to taxonomy in future interpretative work. By the very nature of his exacting task in the arrangement and maintenance of the world's collections of plants the systematic botanist is precluded from obtaining a conversance with much research sufficient to enable him to utilize its conclusions in his systems of classification. Furthermore, much valuable genetical information is of such a kind as to be available to the taxonomists only in the presence of living material which can be grown in controlled conditions over a period of time. Thus, however extensively Gregor's experiments with *Plantago* (1939), and the work of Turrill & Marsden-Jones (1933) on *Anthyllis Vulneraria*, have contributed to our knowledge of the

plants concerned, the conclusion must be drawn that in neither case has the primary end of taxonomy been promoted by the results obtained. Shull (1923) has indicated that he realized to the full the difficulty of utilizing the results of his own interesting studies of *Bursa* for systematic purposes. Indeed, Turrill (1925) has voiced no less distinctly his desire for a 'species-concept' based on generally accepted convenience. The situation is further complicated by the delimitation of a host of new terms defining units within the species, in the original Linnean sense. The appearance of these terms is of course justified by the rapid advances made in the different fields of inquiry, so that the genotype, the phenotype, the ecotype and the ecospecies have each a real significance employed in their appropriate contexts. But in order that any one of these may be correctly used with respect to an individual sample (Turessen, 1922), it is necessary to possess a considerable body of exact information concerning that sample; information, be it remembered, which the man who seeks the name for any given plant will not ordinarily be in a position to supply. In a word, identification must rest on readily observable criteria; and the truth must be faced that all identification is incomplete, varying only in the degree of its incompleteness.

It is exceedingly likely that work in the direction of a fuller understanding of the structural organization of plants, as of animals, will make increasing use of physico-chemical principles and mathematical techniques. How far we are yet from an understanding of the relatively simple life processes of plants is abundantly stressed by Stiles throughout his work on the *Principles of Plant Physiology* (1936); so that it may be long before biologists can find such a physiological explanation of structure as that sought by McLean Thompson with reference to the flowers of angiosperms (1934). Nevertheless, the trend is inescapable; and the master card in the game of evolutionary interpretation has passed from the hand of the formal morphologist to the biologist equipped with a dynamic point of view. In this new phase of the game the taxonomist will find himself increasingly unable to play an effective part.

Unlike other branches of biology taxonomy cannot change with any rapidity. Its accumulated content cannot be set aside, or rendered obsolete, as a result of a single far-reaching discovery. For only by the maintenance of orderly method can the science of systematics serve the needs of those in other fields of biological endeavour. It requires standardized descriptive terms, but insists on the use of judgement in their employment. Modern structural botany abhors such terms, and where terms are employed, prefers experimental evidence to the judgement of the observer. Were it not for the self-evident indications that the 'differences in kind' with which he deals do represent differences in racial history, it would be a comparatively simple matter for the taxonomist to take the alternative path, thereby ceasing to contribute materially to discussions concerning affinities. But when it has been the prerogative of several generations of systematists to pronounce, not so much on systems as on relationships, it is perhaps natural that some should be slow to appreciate the somewhat discouraging portent of the times—that the only really crucial evidence in the case will be withheld for ever from them.

It is possible to envisage a day when systematic botany will be to a large extent divorced from the discussion of phyletic trends, but it is neither likely nor desirable that the entire basis of the subject be removed. Meanwhile it is probable that increasing specialization will be reflected in the development of a much more complete separate 'systematics' of individual organs or other arbitrarily separable parts of plants. The trend witnessed to-day in the work of Record (1919) and others on woods, and with its counterpart in seed structure (Reeves, 1936) and pollen morphology (Wodehouse, 1935), is likely to culminate in the separation of each economically important line of this kind into a utilitarian subscience set apart from interpretative botany. Such separate taxonomies, like the original taxonomy whence they sprang, will flourish only with the employment of clearly defined descriptive terms, used always with judgement. In this connexion may be mentioned the confusion which has led to determined efforts towards standardization of the terms tracheid, fibre-tracheid, and fibre (Reinders, 1935; Bailey, 1936)—employed in the past by different wood anatomists with marked lack of uniformity.

As pointed out by Du Rietz (1930), the shortcomings of floristic taxonomy, as practised at the present day, are quite worthy of reform. He deplores, among other things, the custom of designating as the 'type' of a given species a sheet or a set of specimens, rather than the product of an individual plant. But while his thesis here, as in his appeal for a greater correlation between descriptive herbarium work and experimental field study, is acceptable, he is nevertheless for the most part merely focusing attention on the conflict between the old systematic methods and the modern work on heredity and allied subjects. The very sanctity of the 'type' specimen is in itself eloquent testimony of the conflict. For in looking always *back* to the 'type', and *back* to the original description—accepting therewith the priority rule in nomenclature—the taxonomist turns in the opposite direction from that taken by most of his botanical co-workers. In the interests of systematy he delves into the past; that same past which the experimentalist, intent on progress, is often entitled to regard as virtually obsolete. At every point there is divergence between these two central interests—on the one hand absolute truth, on the other a system, convenient and workable.

IV. TOWARDS ULTIMATE SYNTHESIS

Following an outline of the historical background of the branches of botany here concerned, evidence has been presented of a characteristically modern, dynamic approach to plant structure. In the third section of this article the attempt has been made to indicate something of the position of modern botanical taxonomy, with special reference to what we have termed the more dynamic work on plant structure. The opposition which greeted much of the newer work on anatomy and organography has been traced to an underlying conflict which must always exist between the progressive trend of biological research and the apparently inherent conservatism of utilitarian taxonomy. Furthermore, since it can neither follow the advancing trend in the one direction nor yet abandon the ideal of a natural classification, taxonomy has been depicted poised midway between two alternative courses, neither of which is seen to be wholly acceptable.

To leave the subject at this point would be to omit a consideration of that ultimate synthesis which must effect, in the not too distant future, a *rapprochement* between these somewhat conflicting departments of botanical science. Muriel Onslow's statement (1911) that the plant form is an expression of its chemical constitution may be criticized on the grounds that it assumes a definite constitution in something which is, in the last analysis, merely the locus of continual and organized change. Nevertheless, no part of the physiological processes involved in the life of a plant can be described except in terms of definable structural units. Thus, no treatment of a plant's anatomical organization—however dynamic—can pass beyond the ultimate structural units of current physics and chemistry; and the theoretically complete account of a single plant would be that which included a statement of the physical and chemical constitution of the maximum possible number of different parts of the plant at the shortest possible intervals of time. Clearly no such ideal is attainable with the limitations of the existing language of biologists. The important point emerges, however, that even some future mathematical formula which may be found to express the process of life in a plant will be comprehensible only if applicable to problems that can be stated in terms of structural units. We may change the nature of the descriptive terms or units that we employ, but units of some kind are indispensable for all description.

With these considerations in mind we may return to the conflict between taxonomy and the dynamic approach to plant structure. This conflict is then perceived to be of limited extent. The conflicting viewpoints are not irreconcilable, save in two particular respects. Whereas taxonomy, in serving the community, must follow to some extent the dictates of convenience, research in the direction of ultimate truth is wholly free from this contingency; and while the taxonomist has in the past sought to 'unfold' evolution by the methods of the 'old' morphology, the advancing trend of thought goes to discredit phylogenetic interpretation based exclusively on this type of evidence.

Even while the new approach to structural problems in plants has been manifesting itself there has been an appreciable tendency towards a new synthesis of available knowledge, out of which may be fashioned eventually a more comprehensive taxonomy. Thus, while the subject of systematic anatomy is at least as old as the work of Guettard on trichomes in 1747, it is only within the past few decades that our knowledge of wood structure has advanced sufficiently to permit of the extensive statistical analyses now undertaken. A mass of detailed information has been collected regarding the *processes* concerned in anatomical development. With this new knowledge has come the statistical method in anatomy, which, used with discretion, constitutes a valid check of phylogenetic conclusions reached by other means. Involving a consideration of a large number of cases in the quest for a solution of any particular problem, this method was used in a convincing manner by Sinnott & Bailey in 1916, and has been employed by other workers since that time. After a careful elucidation of the process whereby derivative tissues originated from the cambium it was possible to recognize states of adult tissue arrangement which were correlated with particular types of cambial behaviour; and these states could in turn be defined and used in phylogenetic discussions. In this way both the medullary rays (Kribs, 1935) and the vasa of the xylem (Frost, 1931), after a thorough re-examination from the dynamic standpoint, have submitted to some kind of classification whereby they may be drawn into the orbit of phylogenetic discussion, and at the same time be made available for the purposes of more accurate systematic description.

Inevitably workers in every field of anatomical and organographic botany are confronted with the limitations of biological language. Inevitably, too, their judgement is taxed by the phenomena of structural variation and what in the present state of knowledge appears to be most aptly termed 'parallel development'. Yet neither the extent of variation found in the wood anatomy of *Sequoia* (Bailey & Faull, 1934), nor the most complete resemblance between the vegetative forms of succulents belonging to such diverse families as the Euphorbiaceae, Asclepiadaceae and Cactaceae, will fully justify the agnosticism of Arber or the 'participation theory' of Hayata. For the agnostic view is satisfactory only when it gives place to new and more accurate knowledge.

Vestal (1937), by the study of a single, circumscribed group of dicotyledons—the Guttiferales—has shown how new departments of systematized anatomical knowledge may assist not only the wood systematist, but also those seeking an understanding of relationships. All such evidence may be in the

nature of indirect or 'circumstantial' evidence; but the greater the body of observed facts, the narrower the margin of error. Thus, with the co-operation of many observers, some concentrating on a particular class of evidence—pollen structure, cytological information, wood anatomy and the like, others concerned with the correlation of the different classes of evidence within a given group of plants—the species, genus or family, we may hope to acquire a body of knowledge that will lead us far towards ultimate understanding. At the present time, for example, we are witnessing an elucidation of chloroplasts (Roberts, 1940; Zircle, 1926) which will lead on to an anatomy of chloroplasts, based on the new work and made available to interpretative biology. It matters not whether the advance be referable to a morphological or a chemical class of knowledge, or whether, in the realm of physiological anatomy, it should lie somewhere between these two extremes. In every case it enters the field of accepted fact in terms of structural units. Thus, Uittien (1928) has quite recently drawn attention to the important morphological correlation between palmate venation of the leaf and cymose ramification of the inflorescence among angiosperms. Here, it would appear, a certain *morphological* pattern is sufficiently fundamental to find expression in different organs of a single plant. Again, Stirling (1932–6) concluded from his study of heterostyly in *Primula* that 'each genus or section of a genus' must be characterized by certain 'fundamental relative growth-rates'. Arising out of a series of anatomical observations, came this distinctly *physiological* conclusion. Finally, the extensive work of certain Australian biochemists on *Eucalyptus* oils (Baker & Smith, 1902; Penfold, 1935) may be cited in illustration of a third and strictly chemical line along which evidence can accumulate. Any one of these classes of facts becomes available for the purposes of phylogenetic discussion. Furthermore it is eligible to be a criterion in advancing taxonomy.

The dynamic approach to plant structure is thus seen to be of value when it can replace one terminology by another more closely in accord with observed facts; or when it leads to the rejection of obsolete concepts and the development of others more nearly approximating to truth. In its agnostic element only does it tend to lack constructive power. Similarly, taxonomy may appear static in the face of advancing research, but becomes truly unprogressive only in the hands of those who fail to realize its capacity for slow change. For if a truly 'natural system' represents an ultimate goal, that system will require to be sought through the patient accumulation of facts from every available class of evidence. Since every view of the structure of an organism must be reduced to static terms in the process of description, it follows that no newly acquired facts will be wholly without potential systematic value. Taxonomy, in pursuit of the natural classification, meets thus with all anatomy and all morphology—physical or chemical though these may become—on the common ground of phylogenetic interpretation.

V. SUMMARY

A truly dynamic approach to plant structure has been a matter of the past century. To Linné the species was something 'established in the beginning', and each specimen was made up of distinct 'species' of organ. Some conflict must exist between taxonomy, with its methods dating from Linnean times, and other more recently formed branches of botany. When von Mohl and others had elucidated the finer structure of plants the stage was set for the appearance of Darwin's *The Origin of Species* and Hofmeister's papers on morphology. Much work followed, filling in the details of a map whose outlines Darwin and Hofmeister had sketched. Such theories as that of the carpel took on a new significance. Divergence of opinion showed itself in the objection of Coulter & Chamberlain to the 'hard categories' of the 'old morphologists'. With the appearance of Mendelism a new mode of thought was engendered. The last vestiges of justification for a static view of plant structure vanished. Within recent decades, with ever-increasing specialization there is nevertheless a tendency towards interdependence between various branches of the subject. A feature of the modern outlook is a certain fluidity of concept. The present article deals with evidence of this outlook drawn from morphological and anatomical fields, and its bearing on taxonomy.

Few publications on structural botany have been more controversial than those of such observers as McLean Thompson and Grégoire on the gynaeceum. McLean Thompson carries an emancipated viewpoint to its logical conclusion of almost complete agnosticism. Recent publications indicate that a less rigid attitude is being assumed towards apices, and to the tissues arising from them. In the work of Esau on phloem one is struck by the dynamic approach adopted. The formalized interpretation of the structure of the secondary body enunciated by Jeffrey was opposed by Bailey and others whose critical studies are a further illustration of a dynamic viewpoint. The work of Priestley, Salisbury, and Thoday may be cited as further illustrations of a modern approach. A like trend of thought is apparent in the realm of gross morphology. Arber concludes that leaf and stem are no more than convenient descriptive terms. Thomas arrives at equally revolutionary conclusions. Genetical work has influenced structural concepts where the species is concerned. The new attitude is far from universal, but is well expressed in the stress recently laid by Thoday on the significance of the natural selection of harmonious changes, and the importance of obtaining light on the laws of harmonious development.

The foundations of taxonomy, the primary function of which is systematic arrangement, were laid by Linné. Within recent years systems have appeared purporting to give the 'true' or natural classification. Systematists have thus become interpreters. It is doubtful how far the study of external form and gross morphology can unfold the story of organic evolution. A conflict in fact exists as regards what constitutes valid evidence. Modern views have had repercussions on the working units of the taxonomist. The writings of Guppy and Willis show evidence of mistrust of the Darwinian conception of the mechanism of evolution. Du Rietz goes far in this direction, whilst Lotsy and Hayata provide instances of

extreme iconoclasm. A parallel exists between the agnosticism of Arber in morphology and that of Hayata in taxonomy. Taxonomy, as the science of arrangement, presents a formidable task in itself; its concern with relationships must be secondary. Precise structural units are essential, so that such theories as that of 'acarpous flowering' spell confusion to the taxonomist. Here lies the central conflict between taxonomy and much of the modern work on structure. Taxonomy is faced with the alternative of either undergoing a revolution within itself or ceasing to claim to present more than a convenient arrangement of organisms. Elaborate genetical and other studies do not promote the primary end of taxonomy, if that end is taken to be a workable arrangement of plants. Systematic botany, relatively conservative, may become to some extent divorced from the discussion of phyletic trends. Du Rietz, in pressing for reform in the methods of taxonomy, is focusing attention on the essential conflict between the older systematic methods and the modern work on heredity and allied subjects. Two conflicting interests are concerned, on the one hand absolute truth, on the other a system convenient and workable.

No treatment of the plant's anatomical organization, however dynamic, can pass beyond ultimate structural units. The conflict above noted is thus of limited extent. With increasing knowledge of the processes involved in anatomical development has come the statistical method, as employed by Bailey and others. The agnosticism of Arber and Hayata is in the last analysis unjustifiable. Different classes of evidence may be utilized towards a common end. Advances—no matter in what branch of botanical knowledge—enter the field of accepted fact in terms of structural units, and so become available, alike for phyletic discussion and for use as taxonomic criteria.

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THE ORIGIN OF THE TETRAPODS

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I. INTRODUCTION

The circumstances of the emergence of tetrapod vertebrates involve a number of extremely interesting problems in comparative anatomy, systematics, physiology and the mechanics of locomotion. Some of these problems have received, particularly within the last two decades, a great deal of attention leading to a considerable literature. The last general account (Watson, 1926*a*) summarized very brilliantly a number of fields of palaeontological inquiry up to that time. Since then significant new factors have been the intensive work of Stensiö (fishes), Säve-Söderbergh (fishes and amphibia) and others of their school based on the important discoveries made by various scientific expeditions to East Greenland: Goodrich's clear exposition (1930) of some of the morphological problems; the work of Romer, Gross, Jarvik, Sternberg and Westoll on various crossopterygians; new accounts of dipnoans by Hills, Romer, Holmgren & Stensiö, Forster-Cooper, Watson, Graham-Smith & Westoll; the great increase in precise knowledge of the early tetrapods due to Watson, Säve-Söderbergh, Bystrow, Efremov, Sushkin, Bulman & Whittard, Steen, Romer, Price, White and Wilson; the analysis of the paired limbs by Gregory and his collaborators and by Holmgren, and of the vertebral column by the first-named; the investigation of the homology of dermal bones by Allis, Säve-Söderbergh, Romer and Westoll; and the inquiries made by Sushkin, Romer, Price, Eaton and others into the evolution of the tetrapod ear, and by Hogben & Landgrebe into the optics of the eye in living fishes. Several physiological problems have been more clearly posed during the same time.

It is now generally accepted that the immediate tetrapod ancestry is to be sought in the Devonian fishes included in or related to the groups Crossopterygii and Dipnoi. The majority of investigators regard some part of the former as ancestral (or most closely related) to all known tetrapods, but Holmgren and Säve-Söderbergh have derived the Urodela independently from the Dipnoi. Hence all members of these groups will enter the ensuing discussion. The close relationship between tetrapods, Crossopterygii and Dipnoi has been used by Säve-Söderbergh as the basis of a new taxonomic division, Choanata, to include all three groups; this terminology is not very widely accepted, though Romer has proposed Choanichthyes to include Crossopterygii and Dipnoi. The relationships of Choanata to Actinopterygii, Elasmobranchii and the other gnathostome groups (Aphetohyoidei, Watson, 1937) are not sufficiently close to warrant discussion here.

The essential palaeontological investigations involve mainly the Devonian and the Lower Carboniferous vertebrates. The Crossopterygii comprise three groups: osteolepids, holoptychiids and coelacanths. The earliest known Crossopterygii are species of the osteolepid *Porolepis* from the Upper Siegenian and Lower Coblenzian of Germany, from the upper part of the Lower Devonian (cf. Säve-Söderbergh, 1937*b*) of Spitzbergen, and from the upper part of the Lower Devonian of Russia. The osteolepid section of Crossopterygii persists to the Permian. The holoptychiid section apparently makes its first appearance in the Middle Devonian and persists at least to the end of the Devonian. The coelacanthid section appears first in, or just below, the lower part of the Upper Devonian of Germany (one genus, *Euporosteus*, a very primitive type indeed, was first recorded from the Middle Devonian of Gerolstein, but Stensiö (1937) apparently regards it as of lower Upper Devonian age). I myself possess material of a proto-coelacanth from the earliest Upper Devonian of Canada. The Dipnoi appear first in the Middle Devonian (still earlier records are very questionable, and mostly erroneous).

The earliest large faunas of tetrapods occur in the Lower Carboniferous. Säve-Söderbergh (1932) described the Ichthyostegalia from East Greenland rocks of Old Red Sandstone type which he regarded as uppermost Devonian, but which, as Westoll (1940*a*) has suggested, may be contemporaneous with the lowest Carboniferous elsewhere. Westoll (1938*a*) described an important proto-tetrapod *Elpistostege* from Eastern Canada, from rocks assigned to a basal Upper Devonian age. The other groups of early tetrapods are first known from Carboniferous rocks. The loxommoids and anthracosaurs among Stegocephalia (*sensu stricto*) and forms assigned to the Lepospondyli and Adelospondyli are present in the Lower Carboniferous, Rhachitomi in the Middle Carboniferous (Westphalian), and some at least of the early reptiles can be shown to derive from particular groups of Westphalian and Stephanian 'Amphibia'. It is therefore clear that the early tetrapods show very considerable radiation, and they seem to have undergone rapid evolution in late Devonian and Carboniferous times.

The evidence discussed in this article shows with a

high degree of probability that all known tetrapods can be derived from a single basic structural pattern, which resembles that of osteolepid crossopterygians in so many details that this group is the only possible source of proto-tetrapods. Indeed, there is weighty evidence for a monophyletic origin of known tetrapods from a single osteolepid type in the latter half of Devonian time.

II. FORMAL MORPHOLOGY

(1) *The skull*

In most of the fossil vertebrates here discussed the dermal bones are the only known skull remains; even where present, endocranial parts can usually only be exposed by skilled and laborious preparation. Dermal bones continue therefore to be the most useful parts of the skull in phylogenetic and comparative-anatomical work. Their nomenclature since the first crude attempts at a comparative anatomy has been based on that used in living tetrapods, particularly in *Homo*. The name of a bone should of course indicate its homology. Many difficulties of homology, usually involving a bone present in fossil but unknown in living types, had been perceived and solved at the time of Watson's account (1926*a*), for example, the problem of the squamosal. Probably no grave error in homology of the dermal bones remains *undetected* between typical mammals, living reptiles and the Carboniferous Stegocephalia, though there is still argument about many points. The present chaotic state of nomenclature and homology of dermal bones in Crossopterygii and early tetrapods arises from other causes, and need never have arisen if these fossils had been examined without recourse to extrapolation from other groups, or to theoretical considerations of doubtful validity. The following circumstances contribute to this confusion:

(*a*) The nomenclature of the dermal bones in actinopterygian and crossopterygian fishes is based on the crude comparative anatomy of a century ago, slightly modified to avoid obvious internal confusions (e.g. the replacement of the terms 'postfrontal' and 'supratemporal' by 'dermosphenotic' and 'extrascapular'); much of this long-standing nomenclature is misleading as regards homology.

(*b*) The results of morphological studies on living Actinopterygii have been applied to fossil members of that group, then by strict comparison or more or less uncertain extrapolation to fossil Crossopterygii and Dipnoi, and from these to primitive tetrapods.

In this way a compound error may become obvious, for example, in the nomenclature adopted for primitive tetrapods by Säve-Söderbergh (1932, etc.). (See Table 1.)

Two main attempts to carry a fish nomenclature into the tetrapods have been made: Allis has used a non-committal and cumbersome system, and Säve-Söderbergh has based his work on that of Stensiö. Stensiö (1921, 1923, 1925*a*, 1932, and in Holmgren & Stensiö, 1936) reviewed the homology of crossopterygian and actinopterygian dermal bones in relationship to the latero-sensory system and their mutual spatial arrangement. The work was based largely on earlier work of Allis, especially on *Amia*, and on Pehrson's embryological study (1922) of the same fish. He described especially the unique specimen of *Dictyonosteus arcticus* (U. Dev. Spitzbergen, Stensiö, 1921, 1923, 1932) and *Eusthenopteron foordi* (U. Dev. Canada, 1921, fig. 58), and recognized the following bones in the snout region:

(i) Paired *premaxillaries*.

(ii) Two pairs of *rostrals* (+?median rostral) related to the ethmoid commissure.

(iii) Paired series of *nasals* carrying the supraorbital canal back to the frontals.

(iv) An unpaired *interrostral* and paired *postrostrals* between the nasals, rostrals and frontals.

(v) Two (?three) *supraorbitals* and a *posterior antorbital* bounding the orbit.

(vi) An *anterior antorbital* in front of the last-named, bounding the external nostril with the lateral rostral and some of the nasals.

This description is the basis of much of the modern terminology (cf. Nielsen, 1936; Jarvik, 1937, and the works of Säve-Söderbergh). It is therefore important to realize that Stensiö's figures are greatly idealized, and the figure of *Eusthenopteron* is definitely misleading; and Prof. Stensiö has most kindly demonstrated *Dictyonosteus* to me in Stockholm, and informed me that a new description will be necessary. From the study of normal osteolepids, where many details are known in *Osteolepis* (Fig. 1, and Westoll, 1936), *Eusthenopteron* (Jarvik, 1937; Westoll, 1937*c*, 1940*b*), *Diplopterax* (Figs. 7E, 8A, B), *Thursius*, *Megalichthys* (Fig. 8 C, D) and *Ectosteorhachis* (Westoll, unpubl.), the following comments on this region may be made:

(i) There are no rostral elements separate from the premaxilla (but see Jarvik, 1937, and Westoll, 1937*c*, 1940*b*).

(ii) The nasals are very variable: in some forms there are 6–8 small elements, in others fewer larger bones, along the supraorbital canal; the posterior members may be free or incorporated in the frontals (Westoll, unpubl.).

(iii) 'Interrostrals' cannot be separated from 'Postrostrals'; material of the same species from the same place shows marked variation in number, shape and size of these elements, which have been regarded as anamestic ('space-filling') bones (Westoll, 1936, p. 166).

(iv) There is only one supraorbital and one 'posterior antorbital'.

(v) An 'anterior antorbital' (postnarial, Westoll, 1937*c*) is always present behind and above the external nostril; another bone, known in *Eusthenopteron* and *Megalichthys*, bounds it antero-ventrally (Jarvik, 1937—'lateral rostral'; Westoll, 1937*c*—'prenarial').

The structure of the rest of the dermal skull will be clear from Figs. 1, 3, 4, 6, 7, 8 (also Säve-Söderbergh, 1933; Moy-Thomas, 1935 (figs. 1–3); Holmgren & Stensiö, 1936; Westoll, 1936, 1937 *a*, *b*, *c*, 1940*b*; Jarvik, 1937, etc.). The latero-sensory *canals* have very definite relationships to certain dermal bones; the pit-lines are considered elsewhere (p. 10). The transverse division of the skull-roof is very characteristic, but in some individuals of many genera (*Diplopterax—Thursius in errore*—Jarvik, 1937, fig. 18; *Osteolepis*, *Eusthenopteron*, *Megalichthys*—Westoll, unpubl.) the two 'shields' may show an inter-digitating suture.

Two other groups of Crossopterygii may be recognized.* The holoptychiids differ from osteolepids in the cheek-elongation and cheek-structure and the absence of a separate 'parietal' (Westoll, 1937 *a*, *b*); details of the snout are not yet known. The coelacanths are highly specialized in the nature and arrangement of the latero-sensory system, the bone-pattern of the anterior part of the skull, the absence of a separate 'parietal', of a maxilla, and of a separate quadratojugal, and the specialization of snout, palate and mandible. These peculiarities exclude these groups from the tetrapod ancestry, but the osteolepid group shows detailed cor-

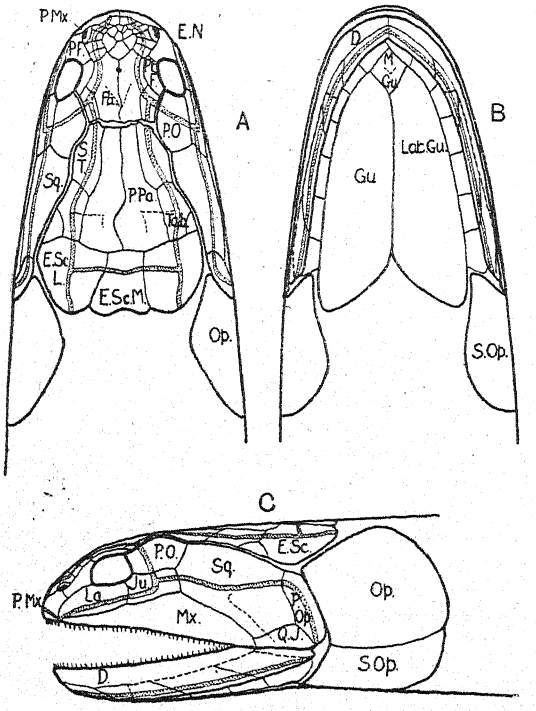

Fig. 1.

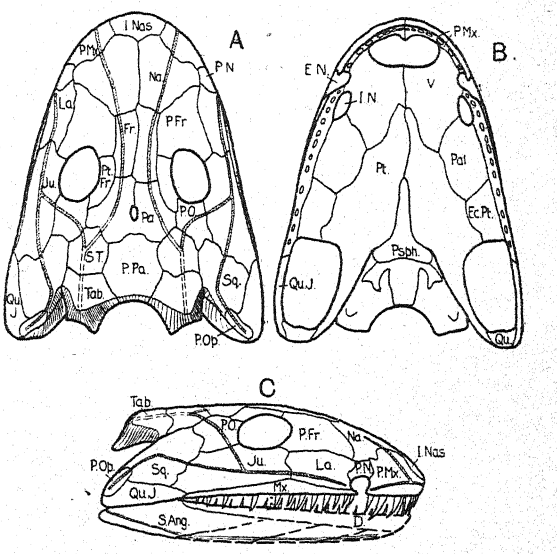

Fig. 2.

Fig. 1. Dermal skull of *Osteolepis macrolepidotus* Ag.; A, dorsal, B, lateral, C, ventral view. Original, based on Säve-Söderbergh (1933). The bone names are those which seem to indicate correct tetrapod homologies.

Fig. 2. Skull of ichthyostegid; A, dorsal, B, lateral, C, palatal view. Composite original drawing, based essentially on *Ichthyostega eigili* S.-S., with details from other forms. Drawn from plates of Säve-Söderbergh (1932). Palatal dentition badly known. Latero-sensory canals partly conjectural.

Explanation of lettering, Figs. 1–3, 6–8

A.C. auditory capsule; *Ang.* angular; *Art.* articular; *b.pt.* basipterygoid process or articulation; *Cor.* coronoid; *D.* dentary; *Ec.Pt.* ectopterygoid; *E.N.* external nares; *E.Pt.* epipterygoid; *E.Sc.* (*L.*, *M.*) extrascapular (lateral, median); *Fr.* frontal; *Gu.* principal gular; *hmd.* hyomandibula; *I.N.* internal nares; *I.Nas.* internasal; *I.T.* intertemporal; *Ju.* jugal; *La.* lacrimal; *Lat.Gu.* lateral gular; *m.c.* mandibular canal; *M.Gu.* median gular; *Mx.* maxillary; *Na.* nasal; *ompl.* oralo-mandibular pit-line; *Op.* opercular; *p.* pineal foramen; *Pa.* parietal; *Pal.* palatine; *P.Art.* prearticular; *P.F.*, *P.Fr.* prefrontal; *pit.* position of pituitary; *P.Mx.* premaxillary; *P.N.* postnarial ('anterior antorbital'); *P.O.* postorbital; *P.Op.* preopercular; *P.Pa.* postparietal; *Pr.N.* prenarial; *P.Sp.* postsplenial; *Psph.* parasphenoid; *Pt.* pterygoid; *Pt.F.*, *Pt.Fr.* postfrontal; *Pt.Ros.* postrostrals; *Qu.* quadrate; *Qu.J.*, *Q.J.* quadratojugal; *s.acc.* sulcus accessorius; *S.Ang.* surangular; *s.d.* sulcus dentalis; *s.m.* sulcus marginalis; *S.Mx.* septomaxillary; *S.Op.* subopercular; *Sp.* splenial; *Sq.* squamosal; *St.* stapes or columella; *S.T.* supratemporal; *Tab.* tabular; *V.*, *Vo.* vomer.

* The Rhizodontidae are not separable from Osteolepidae (Westoll, 1936, p. 168).

respondence to Ichthyostegalia and other primitive tetrapods.

The Ichthyostegalia are important links, but have been in some ways misinterpreted by Säve-Söderbergh (1932), who has carried over some errors into his later works (1934, 1935, 1936, etc.). Dr Säve-Söderbergh has shown me the ichthyostegid material in Stockholm, and I am much indebted to him for his kindness. It seems to me that there are two main sources of confusion:

(i) Identification of cracks in prefrontal and premaxilla as suture and sensory-canal groove respectively, and

(ii) Errors in homology with osteolepid elements; by comparison with abnormalities in osteolepids a scheme of homologies involving differential fusions is set out.

Westoll (1936, 1937*c*) indicated some of these errors, and the discovery of *Elpistostege* (Westoll, 1938 *a*, *b*, 1940 *b* and see Fig. 7F below) provided more direct evidence for a revised system of homology involving great changes in proportions of the entire skull (Figs. 2, 3, 4, 5, 7). The key-elements are the tetrapod parietal (='posterior frontal'+'anterior parietal' of S.-S.) and dermo-supraoccipital or postparietal or interparietal (='posterior parietal'+ elements of 'extrascapular series' of S.-S.), which are clearly homologous with the 'frontal' and 'parietal' of older descriptions of osteolepids. The entire osteolepid extrascapular series is lost. Other important points in the structure of Ichthyostegalia are:

(i) The 'posterior antorbital' and 'anterior supraorbital' (S.-S.) are a single bone, the tetrapod prefrontal. Identification of the former with the stegocephalian lacrimal led to the maxilla of later tetrapods being called 'lacrimo-maxillary' (S.-S. 1935; cf. Westoll, 1937*c*).

(ii) The tetrapod postparietal is unpaired in ichthyostegids (the parietal may be partly affected); this can be matched in several specimens of osteolepids.

(iii) The osteolepid 'dermosphenotic' has no separate existence in *Elpistostege* or ichthyostegids, but is probably fused with the osteolepid 'intertemporal' or possibly with the postorbital (adjacent members of the same lateral-line series—see (vi) below). In other early tetrapods the two elements may remain separate as the tetrapod intertemporal and supratemporal respectively or may (either by fusion, or by reduction of the anterior bone) be represented by a single supratemporal element.

(iv) The snout-bones of ichthyostegids, and hence of other Stegocephalia, have been misinterpreted by S.-S. (1932). The 'rostro-premaxillary', as in many primitive Stegocephalia, was probably curved over the overhanging snout, with the dentigerous margin thickened to form a ledge (cf. some Rhipidistia—Jarvik, 1937; Westoll, unpubl.—and the palatal lamina of Stegocephalia). After examination of the material I am convinced that the 'groove, probably for the ethmoid commissure' (cf. S.-S. 1932, pls. 9–15) is a crack due to crushing, that the 'rostro-premaxillaries' are single elements directly comparable with the osteolepid premaxillary, and that they met on the snout below the 'rostro-interrostral'. The 'rostro-interrostral' is most complete in *I. stensiöi* (S.-S. 1932, pls. 1–4), and its radiation-centre is *not* very anterior; it is almost certainly *not* pierced by the ethmoid commissure (which traverses the premaxillaries) but may receive secondary tubules. The ichthyostegid snout compares exactly with that of Rhipidistia or early Stegocephalia; the 'rostro-premaxillary' is a premaxillary, the 'rostro-interrostral' corresponds to a rhipidistian postrostral or a stegocephalian internasal, and the latero-sensory canals, so far as they are known, correspond exactly (Fig. 2).

(v) The region of the external nostril is newly restored in Fig. 2; secondary incrushing of the marginal parts of premaxilla, maxilla and quadratojugal is very marked in most specimens.

(vi) The tetrapod nasals, frontals and parietals present a more formidable problem. The parietals are undoubtedly homologous with the osteolepid 'frontals' (relations to other bones and endocranium), and the premaxillaries of both are homologous. The tetrapod frontals, nasals, internasals and interfrontals occupy an area equivalent to that covered in osteolepids by the 'nasals' and postrostrals (e.g. S.-S., 1933; Westoll, 1936, 1937*c*, 1938*a*, 1940*b*). Säve-Söderbergh, on 'topographical' grounds, thought that the whole nasal series (1932) or all but the most anterior (1935) had fused with the postrostrals to form the paired tetrapod nasals; in his later account (1935) the anterior 'nasals' were incorporated in the premaxillary, with which the normally separated 'interrostral' sometimes fused. With the recognition of the correct homologue of the osteolepid 'frontal' this view becomes untenable, but the problem of the origin of the large nasal and frontal tetrapod elements, and the fate of the postrostrals, remains. To the solution of this problem work on primitive Dipnoi (Graham-Smith & Westoll, 1937; Westoll, 1937 *a*, *b*, unpubl.) contributes materially. In primitive members the skull-roof is covered by numerous elements of which certain ones are arranged along latero-sensory canals and seem basically to correspond each to one sensory-organ (cf. Fig. 7A–D). The rest of the skull-roof is occupied by general dermal bones, some of which are constantly present (and are generally readily homologized with osteolepid elements) while the remainder are variable anamestic bones (Westoll, 1936, p. 166). Adjacent 'lateral-line' bones often fuse; the resulting element is often broader than single elements. The homologues of the osteolepid 'frontals' are normally two or three separate paired ossicles, but *Fleurantia* (Graham-Smith & Westoll, 1937) shows how fusion of these would lead to a bone very like the osteolepid 'frontal', and with similar relationship to the pineal organ (cf. also

Dipnorhynchus—Fig. 7B). Even among Actinopterygii the frontal and nasal of *Amia*, for example, are each known to develop from several rudiments associated with sensory-organs; the rudiments fuse and by expansion produce the definitive bone (Pehrson, 1922). The growth of large bones after fusion of adjacent lateral-line elements may involve either the invasion of neighbouring areas, or fusion with neighbouring anamestic general dermal bones (cf. Moy-Thomas, 1938). In my opinion (based on much unpublished evidence) the former is more reasonable. Hence osteolepids differ from Dipnoi in lepid postrostrals. The stegocephalian interfrontal is a rare bone, known only in late Carboniferous (e.g. *Ricnodon*, *Mordex*—Steen, 1938), Permian (e.g. *Eryops*) and Triassic (e.g. *Trematosuchus*, *Batrachosuchus*) forms; it is probably a secondary anamestic element (cf. Wormian bone) without phylogenetic significance such as often occurs where three or four large bones meet.* To conclude, the tetrapod nasals and frontals probably include no homologues of osteolepid postrostrals, of which the internasal is a relic; they developed by fusion and expansion of adjacent latero-sensory elements. The common pattern

Table 1. *Homologies of certain dermal bones in osteolepids and primitive tetrapods, with Säve-Söderbergh's interpretation of the latter*

'Orthodox' osteolepid names	'Orthodox' tetrapod names	Säve-Söderbergh's nomenclature
Premaxillary	Premaxillary	Rostro-premaxillary or naso-rostro-premaxillary or naso-rostro-interrostro-premaxillary
Nasal series	Nasal Frontal	Naso-postrostral or naso-postrostro-interrostral Frontal (anterior frontal)
Postrostral series including interrostral	Internasal Interfrontal (?) (Other postrostrals lost)	Interrostral (often incorporated in other bones) Median postrostral (Other postrostrals fused to other bones)
Frontal	Parietal	Fronto-parietal (posterior Fr. + anterior Pa.)
Parietal	Postparietal (= dermosupra-occipital = interparietal)	Parieto-extrascapular (posterior Pa. + median extrascapular)
Antorbital (posterior)	Prefrontal	Anterior supraorbital
Supraorbital	Postfrontal	Posterior supraorbital or supraorbito-dermosphenotic
Dermosphenotic	Intertemporal	Dermosphenotic
Intertemporal	Supratemporal	Supratemporo-intertemporal
Supratemporal	Tabular	Lateral extrascapular
Median extrascapular	(Lost)	(Part of parieto-extrascapular)
Lateral extrascapular	(Lost)	(Lateral extrascapular = tabular)
Lacrimal	Lacrimal	Posterior antorbital
Maxillary	Maxillary	Lachrymo-maxillary
Postorbital	Postorbital	Postorbital or postorbito-dermosphenotic
Prenarial	(? Ventral) septomaxillary	—
Postnarial or anterior antorbital	(? Dorsal) septomaxillary	Anterior antorbital

All other bone-names present no difficulty.

the fusion of a few elements related to the supraorbital sensory-organs to form the osteolepid frontals (= tetrapod parietals). During the evolution of tetrapods a similar process has taken place more anteriorly to produce the frontals and nasals (cf. Fig. 7F). The postrostrals are very variable in osteolepids, and where the 'nasals' are fused to form large aggregates in *Megalichthys* (Westoll, unpubl.) the postrostrals may be restricted to the region homologous with that of the internasal of Stegocephalia. The proportionate elongation and narrowing of the stegocephalian snout probably left less room for the development of anamestic postrostrals. The double internasal of *Orthosauriscus* (Watson, 1926*a*) may be a relic, in so primitive a stegocephalian, of the numerous osteo- shown by *Elpistostege*, Ichthyostegalia and Stegocephalia is strong evidence that all were derived from a single ancestral type in which this process had been completed.

These considerations show beyond reasonable doubt that both the old 'orthodox' homologies and those of Säve-Söderbergh are incorrect both in method and in fact, and application of the same methods in other vertebrates is therefore untrustworthy. Allis (1935, 1936) also attempted a general synthesis of the dermal skull-roof, using a more or

* E.g. bone 'St_x' of S.-S. (1935, pl. 13, fig. 1); 'centroparietal' of *Aphaneramma* (S.-S. 1934, fig. 24); or the 'prepineal' of one individual of *Benthosuchus* (Bystrow & Efremov, 1940, fig. 25).

less non-committal though cumbrous nomenclature. His work was based largely on the highly specialized *Polyodon*, which differs very strikingly from all primitive bony fishes, and led to considerable errors of interpretation. His account need not further be considered.

The conclusions reached above are substantially confirmed by Romer's work on the rhipidistian endocranium (1937, 1941). The equations are shown in Table 1, and the relationship between certain dermal bones and important endocranial structures is shown in Figs. 3, 4. It is clear that there are very great proportional changes between the two groups, providing an admirable example of plastic modification or deformation of Cartesian co-ordinates (cf. also Bystrow, 1935). It is also clear that the tetrapod-osteolepid homologies are essentially simple bone-to-bone equations; only the tetrapod frontals and nasals show modification of the osteolepid pattern, and only the osteolepid extrascapulars, extratemporals and circumpineals have been lost from the skull-roof. The osteolepid skull-roof pattern is the only possible ancestral pattern for tetrapods: those of Dipnoi and other Crossopterygii are specialized in other directions (cf. Westoll, 1937*b*; Stensiö, 1939).

One feature of osteolepids (and of Crossopterygii in general) is often thought to debar them from the tetrapod ancestry—namely, the transverse division of the cranium. In normal osteolepids the anterior part shows a certain possibility of movement (kinetism) in a vertical plane, relative to the posterior part. Though slight in amplitude, this kinetism is associated with a rolling articulation of the autopalatine with the planum antorbitale, a sliding-rolling basi-

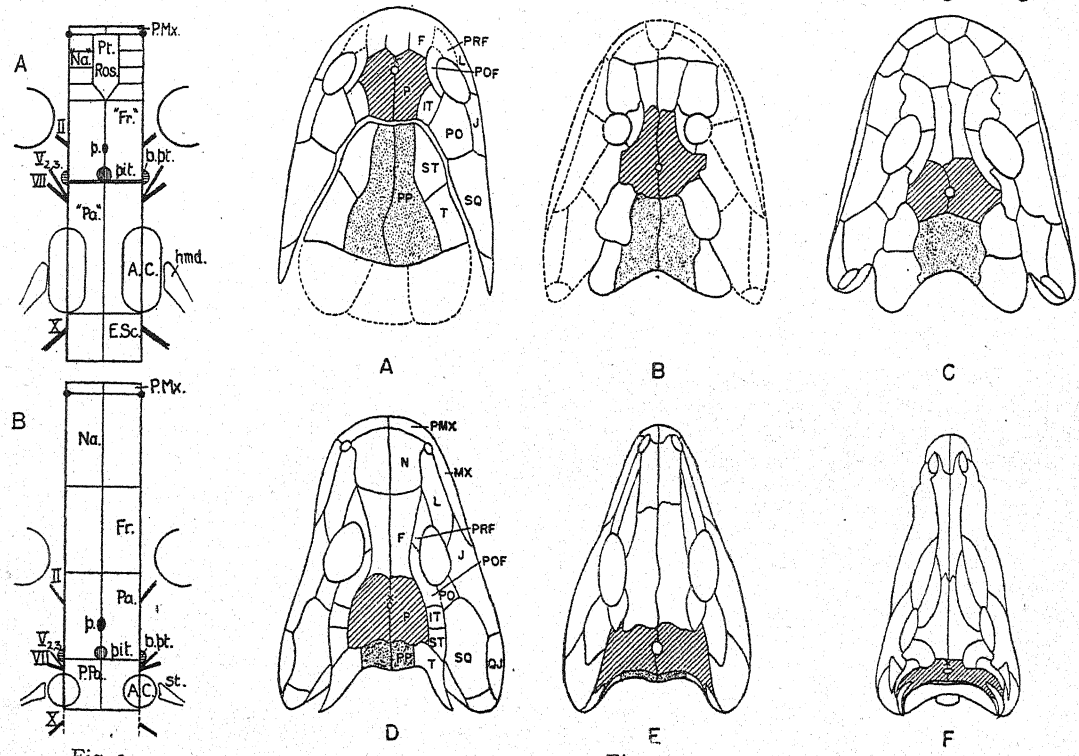

Fig. 3. Fig. 4.

Fig. 3. Diagrams showing relationship of dermal skull-roof bones to important endocranial structures, emergences of cranial nerves, etc., in A, an osteolepid; B, a stegocephalian. Original. For explanation of lettering see Fig. 1.

Fig. 4. Skull-roofs of A, *Osteolepis*; B, *Elpistostege*; C, *Ichthyostega*; D, *Palaeogyrinus* (anthracosaur); E, *Romeria* (cotylosaur); F, *Dimetrodon* (pelycosaur); showing proportional changes in bones of skull-roof, with constant relationship to pineal and pituitary (marked by cross). From Romer (1941). Lettering as in legend to Figs. 1, 2 except that *F*=*Fr.*, *J*=*Ju.*, *L*=*La.*, *N*=*Na.*, *P*=*Pa.*, *POF*=*Pt.F.*, *PP*=*P.Pa.*, *PRF*=*P.F.*, *T*=*Tab.* (From *Journal of Morphology*, **69**, 1.)

This cranial division indicates the retention in most adult osteolepids of embryological units sepa-

pterygoid articulation, and (cf. Aldinger, 1931) movement of the postorbital relative to the skull-roof. These features are not found in tetrapods. In a few individuals of some osteolepid genera, and more frequently in *Eusthenopteron*, the dermal bones of the two regions interdigitate quite firmly (above, p. 80); in *Eusthenopteron* endocranial kinetism seems to have been reduced and the kinetism may thus easily be lost even in osteolepids (cf. Jarvik, 1937; Holmgren & Stensiö, 1936).

rate even in tetrapods, namely, the trabeculae cranii (+polar cartilages) and the parachordals, each with the related capsules and other structures (cf. especially Romer, 1937). It is apparently present in all primitive osteolepids, and determines some details of the dermal-bone pattern; since the tetrapod pattern can only be derived from the osteolepid pattern, it seems to follow that tetrapod ancestors had this trabeculo-parachordal kinetism and have lost it without trace during the great changes in proportion (cf. Figs. 4, 5). The osteolepid conditions would not allow backward growth of the parasphenoid, but this would be possible after elimination of movement; in *Ichthyostegopsis* (S.-S. 1932, pl. 17) a possible intermediate condition, with very slight posterior extension, has been figured (cf. Fig. 2 above). The functional significance of the crossopterygian kinetism is not well understood. Romer (1937, p. 25) has accepted earlier suggestions (e.g. Watson) that it was an adaptation towards absorbing the shocks involved in seizing active prey with the powerful jaws, and this seems quite possible.

The notochord is very large and unconstricted in the cranium of osteolepids, extending as far as the anterior division of the endocranium. It may have played some part in the kinetic mechanism. Stensiö (1932) has suggested that the wide notochord-tunnel and the pit for its anterior end were partly occupied by muscular and other structures, but this is most improbable in view of the closely corresponding size of the vertebral rings. In all known Stegocephalia the cranial part of the notochord, like the remainder, is greatly constricted. It is probable that many of the differences just discussed are correlated with the great increase in the facial part of the skull, and with the development of a strong neck-musculature and neck-joint in tetrapods.

Insufficient is known at present about the endocranium of early tetrapods to allow any thoroughgoing comparison with osteolepids (e.g. *Ectosteorhachis*), though Romer (1937, 1941) has recently made most suggestive comparisons of certain details. It should be noted that Sternberg's description (1941) of the cranium of *Eusthenopteron* takes little account of extensive crushing of his material.

The structure of the dermal bones of the cheek, palate and mandible is practically identical in osteolepids and primitive tetrapods; from this pattern the holoptychiids, coelacanths and Dipnoi show increasingly wide divergence. The Ichthyostegalia are unique among Stegocephalia in retaining a preopercular, which seems to be completely reduced in other tetrapods. In Stegocephalia the jugal normally meets the quadratojugal, and the maxilla tapers posteriorly, while in osteolepids the maxilla may be deeper posteriorly and the jugal and quadratojugal do not meet. Slight differences in proportion and relative growth account for these differences. The primitive condition of the maxilla, in tetrapods and osteolepids, is a shallow bone with an anterior radiation centre; the anterior deepening in tetrapods and the posterior expansion in, e.g., *Megalichthys*, are divergent developments.

The dermal palates of osteolepids and primitive tetrapods are most strikingly similar (Figs. 2C, 6A–C), as already shown by Watson (1926*a*) and confirmed in detail by much other work (Jarvik, 1937; Westoll, unpubl.). The relationship of the internal nares to the vomers* and dermo-palatines is the same in both groups. The primary palate in osteolepids articulates with the posterior face of the nasal capsule (ethmoid articulation), and with the basipterygoid process (basal articulation); above the latter there is a short upwardly directed epipterygoid, and behind this the dorsal margin of the palate is closely applied to the side-wall of the cranium,

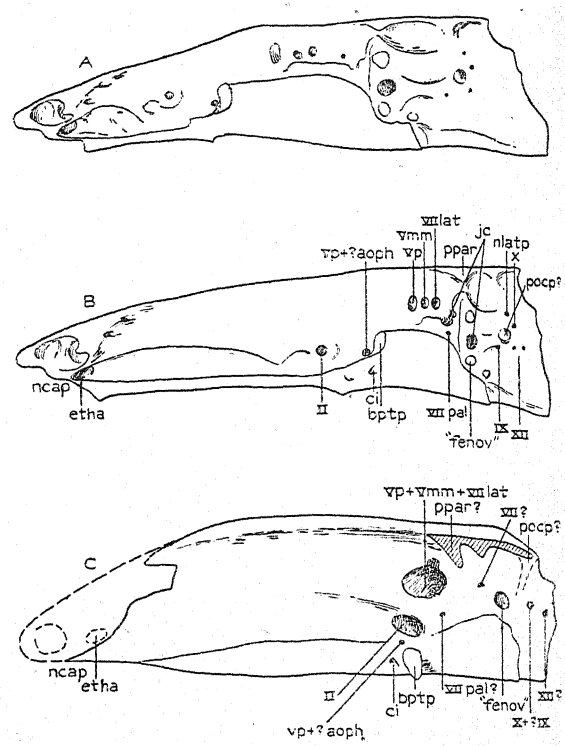

Fig. 5. Differences in proportions between osteolepid and stegocephalian. From Romer (1937). 'A, Side view of the brain-case of *Megalichthys*" (Osteolepid)" so modified, by the fusion of the two moieties and the extension of the parasphenoid backward over the exposed notochordal area, as to eliminate the kinetic specializations. B, The same, further modified in an amphibian direction, by a change of proportions, to introduce an elongated "sphenethmoid" region. C, Diagram of the side view of a primitive embolomerous amphibian, from Watson's figures and descriptions.' Supposedly homologous foramina and structures can easily be followed. (From *The Bulletin of the Museum of Comparative Zoology*, **82**, 1.)

* The nomenclature of these elements has been confused; Parrington & Westoll (1940) showed that 'prevomer'=mammalian vomer.

forming an incipient otic process. In tetrapods ethmoid, basal, ascending (developed from the epipterygoid = metapterygoid of osteolepids) and otic articulations are present; possibly the relative shortening of the skull behind the basipterygoid processes is correlated with the new, firmer union.

The structure of the mandible is also almost identical in early tetrapods and in osteolepids* (Fig. 6 D, E, F); that of holoptychiids is similar, but the coelacanth jaw is greatly specialized. It should be mentioned that the osteolepid mandible, like the cheek and the anterior and posterior cranial-roof shields, may be covered externally with continuous cosmine which often obliterates sutures; the oralo-mandibular pit-line has often been mistaken for a suture.

The teeth, alike in osteolepids, holoptychiids and Stegocephalia, seem to be of three kinds: namely, smaller conical teeth borne on the dentary, maxillary and premaxillary; larger conical teeth borne in couples on the coronoids, vomers, dermo-palatines and ectopterygoids; and a general granulation borne on the palate and lingual surface of the mandible. The large teeth (Watson, 1926*a*; Parrington, 1936) are well known to be of basically similar structure with much-folded dentine (hence 'labyrinthodont'); recently Bystrow (1938*b*, 1939*b*) has discussed them afresh, described their resorption and confirmed and amplified older views of their affinity.

It is therefore clear that primitive tetrapods such as Ichthyostegalia could have been derived only from osteolepid Crossopterygii, on the evidence of their skull-pattern. The question of a polyphyletic origin has recently been raised, and it is necessary to examine the known groups of early tetrapods. The loxommoids, adelospondyls, lepospondyls and probably anthracosaurs are present in Lower Carboniferous rocks, and even ichthyostegids are probably post-Devonian (Westoll, 1940*a*). This leaves ample time for minor divergent evolution in skull-pattern since the early Upper Devonian (*Elpistostege*).

Säve-Söderbergh has emphasized one such difference, namely, that the squamosal and skull-table may be loosely attached (e.g. in osteolepids, *Elpistostege*, anthracosaurs) or firmly fixed by suture (e.g. Ichthyostegalia, *Otocratia*, loxommoids, labyrinthodonts, phyllospondyls, etc.). This is clearly a very minor feature, possibly adaptive.

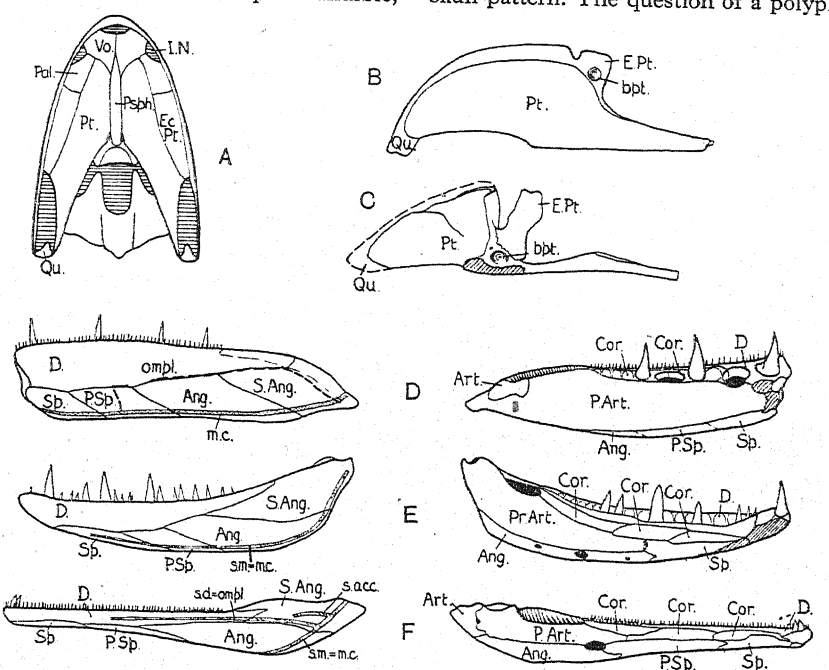

Fig. 6. Palates and mandibles of osteolepids and Stegocephalia. A, Palate of *Eusthenopteron*, an osteolepid. After Watson, with modifications from Jarvik, Holmgren and Stensiö, and new observations. B, Palatal complex of osteolepid. Sketch embodying conditions in *Eusthenopteron* and *Megalichthys*. Original. C, Posterior part of palatal complex of the stegocephalian *Benthosuchus*, after Bystrow & Efremov (1940). The only ossification in the cartilaginous palate is the epipterygoid. D, Outer and inner views of mandible of the osteolepid *Megalichthys*, slightly modified, after Watson (1926*a*). E, Outer and inner views of mandible of the primitive stegocephalian *Orthosauriscus*, after Watson (1926*a*). F, Outer and inner views of mandible of the advanced stegocephalian *Benthosuchus*, after Bystrow & Efremov (1940).

* In *Eusthenopteron* Jarvik (1937) described a separate tooth-bearing plate applied to the prearticular; this observation needs confirmation before its significance can be assessed.

The tetrapod intertemporal-supratemporal region also offers difficulties. Separate intertemporals may occur in loxommoids and some Rhachitomi (e.g. *Dendrerpeton*, *Cochleosaurus* and *Trimerorhachis*), and also in anthracosaurs; they are absent in other members of these groups, in *Otocratia*, Ichthyostegalia and in *Elpistostege*. The intertemporals seem to show marked reduction in some loxommoids (cf. figs. in Watson, 1926*a*, *b*; S.-S., 1935) and this is possibly their general fate, but it is quite possible that in other forms they have fused with the supratemporals. During the obliteration of the transverse cranial division of an osteolepid the homologous elements ('dermosphenotic' and 'intertemporal' of recent usage), being adjacent members of the same sensory-canal series, could easily fuse, and would thereby stiffen the joint-region. It is perhaps permissible to regard them as having been for some time after this stage potentially separate, but often 'phenotypically' fused, and to suppose that gradually a genotypic condition of a single ossification became habitual. It is also possible that the 'dermosphenotic' may in some forms have fused with the postorbital.

Säve-Söderbergh (1934, 1935, 1936) has noted that the anthracosaur 'Stegocephalia', *Seymouria* and early reptiles differ from Ichthyostegalia, loxommoids and labyrinthodonts in the structure of the skull table. In the latter group (to which osteolepids and *Elpistostege* may now be added) the tabulars are small and are cut off from the parietals by the postparietals (dermo-supraoccipitals) and supratemporals; in the former the tabulars are much larger and meet the parietals. Säve-Söderbergh has regarded this difference as due to differential fusion of elements, for which however no evidence can be obtained from the relationship of dermal bones to endocranial structures. The first group seems merely to show exaggerated shortening of the otico-occipital region, with retention of an important tabular, which, in anthracosaurs, is clearly correlated with a different attachment of the shoulder-girdle. The effect is in some cotylosaurs and higher reptiles enhanced by the progressive embayment of the skull-table by the cervical musculature. Säve-Söderbergh has used this difference in his classification of early tetrapods (1934, 1935) into Reptiliomorpha (anthracosaurs and reptiles) and Batrachomorpha. The anthracosaur pattern is shown by *Seymouria* and all primitive reptiles (with the possible exception of the badly known *Pantylus*, cf. S.-S. 1935, pp. 109–11). The *Melanerpeton* group of phyllospondyls shows the same pattern (Steen, 1938) and may include juvenile anthracosaurs; Romer (1939) suggests that 'Phyllospondyls' are essentially juvenile or larval Stegocephalia, the *Branchiosaurus* type being related to labyrinthodonts. Possibly some labyrinthodonts became mature while still small, and developed along somewhat different lines; this would accommodate both Romer's (1939) and Watson's (1940) views on the nature of phyllospondyls.

The two remaining 'orders' of early fossil Amphibia are the Lepospondyli and the Adelospondyli. Steen (1938) reviewed the former, and showed that the 'Microsauria' are mostly *incertae sedis*, and that the type-species of the original family of the group is an adelospondyl. The remaining groups are Nectridia and Aistopoda; the skulls of both show the characteristic tetrapod pattern (with frontals and nasals), but the aistopod skull is highly aberrant. The nectridian skull-pattern is somewhat similar to that of anthracosaurs.

The adelospondyls are so variable in cranial structure that the group may be heterogeneous (cf. Watson, 1926*b*; Steen, 1931, 1938). All show some easily recognizable modification of the primitive tetrapod pattern, and there is no evidence to suggest that any member was independently derived from fishes. The most important members of the group for this discussion are:

(*a*) The late Dinantian *Adelogyrinus* and *Dolichopareias*, which are nearly the earliest known Amphibia except the ichthyostegids, and may be derived from ichthyostegids or *Otocratia*-like forms.

(*b*) The late Carboniferous *Cocytinus* (Romer, 1930; Steen, 1931) and the early Permian *Lysorophus* (Sollas, 1920).

(*c*) The late Carboniferous (mainly Westphalian) *Hylonomus*, *Microbrachis*, *Hyloplesion* (cf. Steen, 1934, 1938; Watson, 1940).

The second group comprises elongated and rather highly specialized forms; it has been thought on various occasions to be ancestral to urodeles or Gymnophiona, with which it shares many palatal details, but it seems to me to be already too specialized to lie on the direct urodele line. Steen (1938) has pointed out that the lysorophids show how a gymnophionid-urodele type of palate could arise quickly from a more generalized ancestor.

The *Microbrachis* group is equally interesting, with an anthracosaur-type skull-table (*Hyloplesion*). The otic notch is reduced, and the supratemporo-intertemporal region tends to be greatly reduced, while the post parietals and tabulars tend to lie on the occiput. The group is astonishingly similar to captorhinomorph cotylosaurs, to which it may be related (Westoll, 1942 *a*, *b*).

There is therefore no good evidence from the skull-pattern for any but a narrowly defined group of proto-tetrapods, originating from osteolepid Crossopterygii (see Section IV). Most workers now accept the derivation of the reptiles and Anura from groups already discussed, but Wintrebert, Holmgren and Säve-Söderbergh have suggested that the urodeles are more closely related to Dipnoi.

The origin of the Anura has recently been illuminated by Piveteau's discovery (1935) of the Eo-Triassic *Protobatrachus* (cf. Watson, 1940), which has a frog-like skull with certain labyrinthodont traits but retains a short tail and has no urostyle. Säve-Söderbergh (1936) showed that labyrintho-

donts and frogs are closely comparable in anatomy, and Watson (1940) has shown that the late Carboniferous *Amphibamus* and *Miobatrachus* may plausibly be regarded as morphologically ancestral to the Anura. The affinities are apparently with the non-anthracosaur group.

The geological history of urodeles is badly known (early Cretaceous—Recent). The skull-pattern is based on the normal tetrapod type, with frontals, nasals and septomaxillaries. Recently Bystrow (1939*a*) has shown that the hyobranchial apparatus and other features of the neotenic labyrinthodont *Dvinosaurus* can only be compared with those of urodeles. Wintrebert's work on the palate (1910, 1922*a*, *b*) is quite consistent with the derivation of the group from more 'normal' Amphibia; the breakdown of the outer dermal arcade of the upper jaw, and the development of the broad parasphenoid and the curious pterygoid, may all be correlated with the forward inclination of the suspensorium and a marked change in the arrangement and mechanics of the masticatory musculature. Possibly such a change would allow the derivation of urodeles from branchiosaurs, to which they are so closely similar in body-shape, but failing them it is still unnecessary to go outside the smaller Carboniferous and Permian Amphibia for the probable ancestry of urodeles.

Finally it may be noted that Bystrow (1935) has used the ornament of the dermal bones of Stegocephalia in the analysis of plastic changes during growth, which he also extended to other early vertebrates. Orlov (1939) has noted that the arrangement of the 'ribs' of the ornament may be of mechanical significance.

(2) *Dermal bone histology*

No recent comparative account of broad scope deals with this question. It has been asserted by Hartmann-Weinberg that the dermal bony scales of the Permian seymouriamorph *Kotlassia* were provided with cosmine, but this has been opposed by Bystrow's work (1940). Bystrow has also contributed notably to our knowledge of the structure of the dermal bones in some labyrinthodonts (1935); sections of dermal bones of very large osteolepid Rhipidistia (without cosmine) from the Upper Devonian of Scotland have been made by myself and show broadly comparable structures.

(3) *Latero-sensory system*

Many recent authors have accepted a fundamental morphogenetic connexion, in primitive bony fishes, between some parts of the latero-sensory system and certain dermal bones, so that homology of the latter can be established. The main basis of this view is the work of Allis on living fishes, and in recent years much emphasis has been laid on Pehrson's embryological study of *Amia* (1922) (cf. also Hammarberg, 1937; Aumonier, 1941). In most living teleosts the ancient character of the dermal bones has become so greatly modified that it is doubtful whether the conditions shown by them are of wider application than to the fishes concerned (cf. Moy-Thomas, 1941; Westoll, 1937 *a*, *c*, 1941). In Devonian Dipnoi and Rhipidistia the latero-sensory system seems to be of great value in establishing dermal-bone homologies. Much unpublished work of my own is incorporated in this Section.

Among Dipnoi *Dipnorhynchus* (Hills, 1933; Romer, 1936*b*) is apparently the most primitive form; as here interpreted (Fig. 7B) the supraorbital sensory-canal does not 'degenerate' posteriorly to a pit-line and is apparently separate from the infraorbital canal. In *Dipterus* (cf. Fig. 7C) the anastomosis of the supraorbital and infraorbital canals, and the reduction of the posterior part of the former to form the 'anterior pit-line', have already occurred (cf. the phylogeny of actinopterygians and the ontogeny of *Amia*), and in later Dipnoi the whole of the supraorbital canal behind the anastomosis is similarly reduced.

The earliest osteolepids already show conditions like those of *Dipterus* or later Dipnoi (Fig. 7E, F), with the supraorbital canal behind the anastomosis 'reduced' to a pit-line. In both Dipnoi and osteolepids the canals proper traverse the dermal bones through or very near their centres of radiation. Nielsen's theory (1936), based on certain coelacanths, is unsatisfactory, and I hope to discuss it elsewhere.

In the Ichthyostegalia the canals are also enclosed, opening by tubules in the usual piscine fashion. Säve-Söderbergh (1932) has based his analysis of the system on a mistaken series of bone-homologies. The probable course of the canals is shown in Fig. 2 (see also p. 81). Säve-Söderbergh (1932, pl. XVI, fig. 2) figures a fragmentary skull-roof which appears to show paired grooves in the appropriate position for an anterior pit-line.

In all other Stegocephalia and the smaller Carboniferous Amphibia the latero-sensory system formed only superficial sulci ('slime-canals') on the dermal bones.* These grooves show a remarkable independence of the dermal-bone pattern. Thus there is marked lateral shift of the supraorbital sulcus from its primitive course; it may run along sutures, or may migrate far laterally, as in *Trematosaurus* where it traverses premaxillary, nasal, lacrimal, prefrontal, frontal, postfrontal, or in *Metoposaurus* where it does not cross the frontal at all. Similarly the courses of the 'main lateral line—infraorbital' unit and the jugal canal resemble those of osteolepids in primitive Stegocephalia; but in *Mastodonsaurus*, for example, the former lies in the suture between (tabular + supratemporal) and squamosal, and in many forms this sulcus traverses the maxilla, while the jugal sulcus may run entirely on jugal and quadratojugal. Similarly the occipital cross-commissure is often

* A possible exception is the late Carboniferous *Stegops* (Romer, 1930; Steen, 1931), where the apparently enclosed canals show a unique secondary condition.

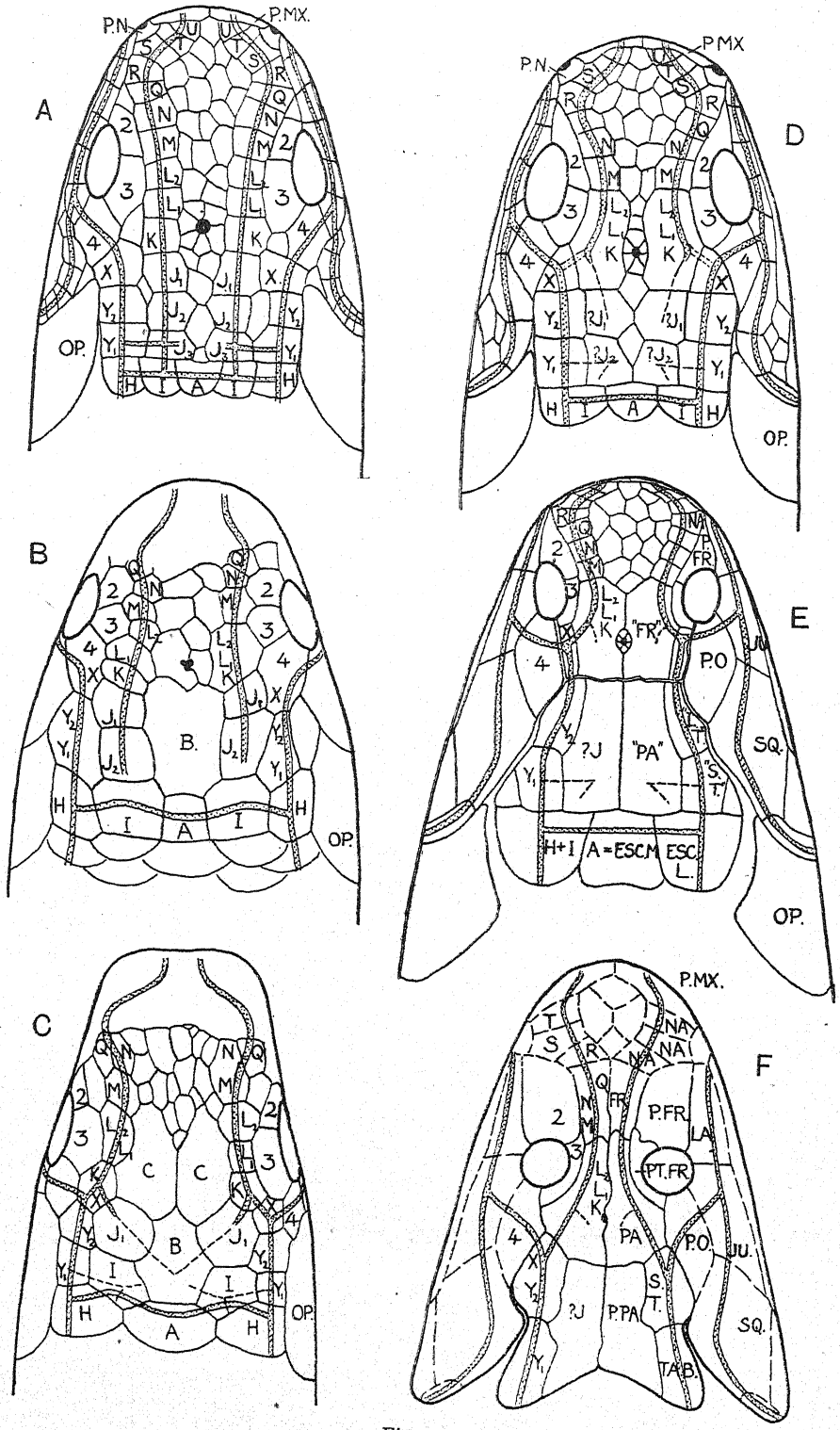
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Fig. 7.

found on the tabulars and postparietals. These displacements are, on the whole, progressive; they are most developed in Triassic forms. They may show distinct differences in species of the same genus (e.g. *Lyrocephalus*, S.-S. 1935) or on opposite sides of the same individual (e.g. *Aphaneramma*, S.-S. 1936, fig. 31 B). It is therefore clear that there was no fundamental morphogenetic connexion between dermal-bones and latero-sensory system in these Stegocephalia. A working hypothesis to account for this has been developed (Westoll, 1937*a*, p. 379, 1940*b*, 1941).

These latero-sensory grooves of Stegocephalia are in many ways analogous to 'pit-lines' in fishes. These 'pit-lines' are normally almost completely reduced in Stegocephalia. Apart from the rather common 'sulcus dentalis' (Bystrow & Efremov, 1940) on the mandible, probably homologous with the oralo-mandibular pit-line of osteolepids, the only remnant known to me is the sulcus representing the vertical pit-line of the cheek in *Palaeogyrinus* (Watson, 1926*a*). In some later osteolepids a pit-line may be represented by a series of short parallel grooves, an apparently meaningless multiplication possibly implying loss of morphogenetic control of an obsolescent structure. The same phenomenon is shown in some of the sulci of Stegocephalia, e.g. the vertical pit-line of *Palaeogyrinus* (Watson, 1926*a*), and on the skull-roof of *Lyrocephalus*, *Aphaneramma*, etc. (S.-S. 1935, 1936). The 'sulcus accessorius' on the surangular of *Benthosuchus* (Bystrow & Efremov, 1940) may be due to similar duplication, or possibly to the ventro-lateral migration of the 'vertical pit-line' over the skin behind the angle of the mouth.

Some of Säve-Söderbergh's interpretations of the system in Stegocephalia are based on misunderstanding of its nature. The lateral-lines were almost certainly not enclosed canals; there can therefore have been no anastomosis of primary tubules, hence no 'postnasal connexions'. The sulcus in some loxommoids so named by him is probably part of a loop of the infraorbital canal on the lacrimal, isolated and distorted by the development of the preorbital fenestra.

The distribution and histology of the degenerate latero-sensory system in living Amphibia are discussed by Escher (1925). It is very interesting that the reptilian 'Tastflecken', and tactile hairs (vibrissae, etc.—Tasthaare in German) on the body of such mammals as *Heterohyrax* occur along almost identically arranged tracts as compared with the amphibian latero-sensory organs (Dabelow, 1927, p. 327 and Escher, 1925 give discussion and literature). Broman (reviewed by Escher and by Dabelow) has also suggested that the mammalian mammary glands may be related phylogenetically to parts of the latero-sensory system.

(4) *Ear and 'stapes'*

I have discussed (in an article to be published elsewhere) the hyomandibular of some Rhipidistia with reference to the origin of the columella auris or stapes, and also the origin of the tympanic diverticula of tetrapods. Some of the conclusions have influenced later parts of this article. It is only necessary here to note that the peculiarities of the ear-region of Urodela, Gymnophiona and Anura strongly suggest a common ancestry from labyrinthodont Stegocephalia or their small 'phyllospondyl' relatives. (See Addenda, p. 98.)

(5) *The nasal region*

The osteolepids and early tetrapods show marked resemblances in the position and openings of the nasal capsules, which are moderately well separated. In typical forms of both groups each capsule has two openings, the external being laterally placed on the snout, the internal opening on the palate, normally between vomer, maxilla and dermopalatine. Stensiö (1932, and in Holmgren and Stensiö, 1936) figured Lower Devonian *Porolepis*-like forms which seem to show *two* external nasal openings. I have seen the original material and believe it was misinterpreted by Stensiö; the 'posterior external nasal opening' corresponds to a notch in other forms which receives the lacrimal. Ichthyostegalia (S.-S. 1932) have the internal and external nares practically confluent (Fig. 2), and in many early osteolepids (Westoll, unpubl. and cf. Fig. 8 A, B) the same is true. In later osteolepids and tetrapods (Fig. 8 C–I) the premaxilla meets the

Legend to Fig. 7.

Fig. 7. Possible stages in evolution of choanate skull-roof patterns. A, Hypothetical ancestral condition. Numerous small elements related to latero-sensory organs, special bones related to orbit and external nostril, and variable anamestic elements. B, Primitive dipnoan, *Dipnorhynchus*. Modified from Hills (1933). Broader skull-roof, increase in importance of median anamestic series, fusion of adjacent latero-sensory bones. C, Early dipnoan, *Dipterus*. From Graham-Smith & Westoll (1937). Continuation of same changes, anastomosis of supraorbital and infraorbital canals and degeneration of posterior part of former (anterior pit-line), with correlated loss of some latero-sensory bones and forward migration of the extrascapular I. D, Hypothetical stage leading to osteolepid condition. Fusion of latero-sensory elements to form osteolepid 'frontals', anastomosis of supraorbital and infraorbital canals and degeneration of posterior end of former. E, *Diplopterax*, showing continuation of processes begun in D, with elongation of cheek and jaws, and posterior part of skull-roof. Great reduction in anamestic bones between 'frontals'. F, *Elpistostege*, attempted reconstruction. The snout is entirely hypothetical; it is suggested that the most anterior members of the osteolepid 'nasals' are still separate and not yet fused to form tetrapod nasals. The system of lettering the bone-elements is essentially that used by Forster-Cooper (1937) and Graham-Smith & Westoll (1937) in the study of Dipnoi.

maxilla below the external nostril, which is carried up on to the snout (cf. *Orthosauriscus*, Watson, 1926*a*).

The details of the snout of holoptychiids are unknown. The conditions in coelacanths are remarkably specialized and are still little known. The living *Latimeria* (Smith, 1939) gives only a partial answer to the problems raised by earlier work, and I hope to deal with them on another occasion; they do not affect the present discussion. In Dipnoi the nasal capsule has two openings—an anterior, external one forming a notch in the upper lip, and a posterior, internal opening on the palate lateral to the vomer-pterygoid junction. Broman (1939) showed that the choanal canal in living Dipnoi is probably not habitually used for respiration, but the link, based on the choanae, between Dipnoi, Rhipidistia and tetrapods (e.g., S.-S. 1934; Romer, 1941) is certainly justified.

It is worth pointing out that the ontogenetic development of the face, even in mammals, closely parallels the general phylogenetic evolution of osteolepids and Stegocephalia (Fig. 8 K–M).

The identification of the homologue of the tetrapod septomaxillary in osteolepids has recently been reviewed by Jarvik (1937), who describes the nasal region of *Eusthenopteron*. Westoll (1937*c*, 1940*b*) has criticized the nomenclature used by Jarvik; the bone called by him 'lateral rostral' is not a rostral and is better termed prenarial, and the 'anterior antorbital' is now called postnarial. The postnarial is present in all adequately known osteolepids, and has often been recognized as the homologue of the septomaxillary. The prenarial bone is known only in *Eusthenopteron* (Jarvik, 1937; Westoll, 1937*c*, 1940*b*), in *Megalichthys* (cf. Fig. 8 C, D; also Westoll, unpubl.; Holmgren & Stensiö, 1936, fig. 272*c* is inaccurate) and in *Ectosteorhachis* (Westoll, unpubl.). In external view the postnarial lies above and behind the external nostril, and meets the fronto-nasal series, premaxilla (except in *Megalichthys* and *Ectosteorhachis*), the prefrontal and often the lacrimal (in *Eusthenopteron*, perhaps also in *Megalichthys* and *Ectosteorhachis*). The prenarial lies below the external nostril, has broad contact with the premaxilla and meets the lacrimal; in most cases it meets the nasal series. In ichthyostegids (S.-S. 1932) the 'anterior antorbital' of that author is dorsal to the external nostril, and meets the premaxilla, nasal and lacrimal; except in *I. stensiöi* it is just excluded from the prefrontal, and may touch the maxilla. The bone is almost certainly homologous with the osteolepid postnarial, and by direct comparison is clearly homologous with the septomaxillary of many Stegocephalia, where the exclusion of the bone from the prefrontal, due to the lengthening of the snout, is carried much further. This conclusion is opposed by Jarvik (1937,

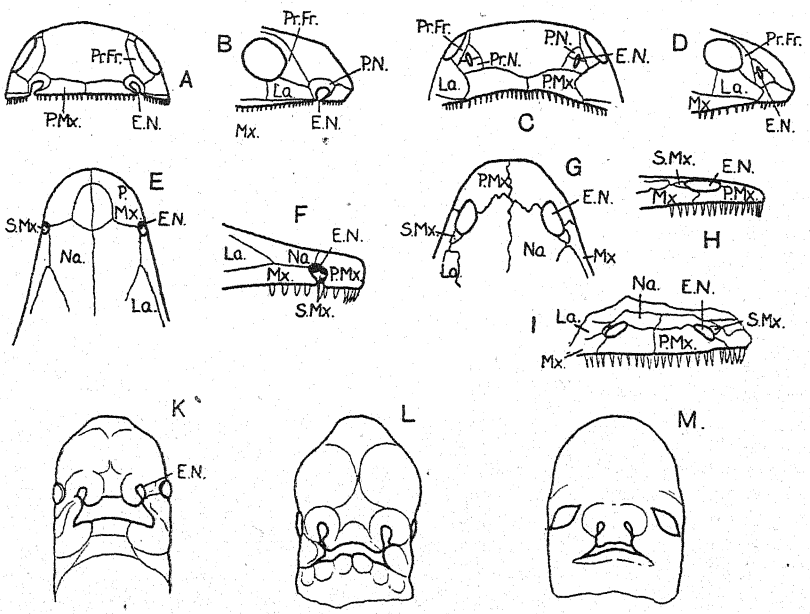

Fig. 8. The external nares. A, B, Anterior and lateral views of snout of *Diplopterax* (Mid-Devonian). Semi-diagrammatic. C, D, Similar sketches of *Megalichthys* (Westphalian), showing dorsal migration of nostril. Semi-diagrammatic. E, F, Dorsal and lateral views of snout of *Orthosauriscus* (Westphalian), a loxommoid. From Watson (1926*a*). Nostril nearly marginal (cf. ichthyostegid, Fig. 2), ventral septomaxillary (?prenarial). G, H, I, Dorsal, lateral and ventral views of snout of *Lyrocephalus* (Eo-Triassic), a stereospondylous labyrinthodont. Taken from plates of *L. johanssoni* S.-S., Säve-Söderbergh (1935). Nostril has migrated dorsally; postnarial septomaxillary. K, L, M, Three stages in formation of human face; K, 8 mm. (after Peters); L, 10 mm.; M, 15 mm. (both after His). Note similar dorsal migration of external nares.

p. 119), who showed that the cavum nasi in *Eusthenopteron* is partly subdivided by a nearly horizontal 'processus intermedius', to the dorsal surface of which an inturned lamina of the prenarial is applied. He therefore regarded this bone as the septomaxillary because the septomaxillary in Anura has similar relationships to a 'processus intermedius'. The problem of the septomaxillary needs to be examined further, both from palaeontological and embryological aspects. It is not impossible that two quite distinct elements have been confused under this name in tetrapods. It is very illuminating to compare the bone in *Miobatrachus*, where it is ventral to the nasal opening and has indeed a palatal exposure, and in *Eugyrinus*, where it is in the position of most normal Stegocephalia (Watson, 1940; cf. also Fig. 8 E–I). Nothing is known of the relation between septomaxillary and Jacobson's organ in very early tetrapods.

An interesting feature of the internasal region in many Stegocephalia is the presence of a median opening or fenestra between the premaxillaries. This is called the 'interrostral fenestra' by Säve-Söderbergh (1935, pp. 22–9), but that name is based upon false homology of a dermal bone, and it is better to return to the older term 'rostral opening' (or fenestra). Säve-Söderbergh also discussed the nature of this and other fenestrae in the dermal bones, and concluded that they were related to an intermaxillary gland, and to paired naso-labial glands; von Huene and Nopcsa had previously come to similar conclusions. Wilson (1941) has contributed notably to our knowledge of the intermaxillary gland in Stegocephalia by his description of *Buettneria*. In this form there are three sets of openings supposed to be related to the gland: an external rostral opening, and anterior (multiple) and posterior openings on the palate. The conditions on the palate thus combine the peculiarities of certain Urodela and Anura; this removes the value of another character used by Säve-Söderbergh to separate those living Amphibia. It is interesting that Jarvik (1937) found anterior and posterior median openings between the vomers of *Eusthenopteron*, which seem to have been related to a similar intermaxillary gland.

(6) *Gills and lungs*

The number of branchial arches in Rhipidistia is not certainly known; there were at least four, probably five in most forms (Watson, 1926*a*, fig. 36; Sternberg, 1941). *Latimeria*, the living coelacanth, may have only four (Smith, 1939). Sternberg identifies (with some doubt) 'infrapharyngobranchial', epibranchial, ceratobranchial and hypobranchial elements in *Eusthenopteron*, but in other material other workers have found only two elements in each arch, as in coelacanths. A prominent groove for the afferent artery is usually found on the ventral face of the ceratobranchials. It is very probable that the hyobranchial slit was a functional gill opening. In all known Amphibia the hyobranchial slit has lost this function, and there are usually three gill slits. Some of the implications of this are mentioned elsewhere (p. 94, and Westoll, unpubl.).

It is well known that coelacanths had large dorsal air-bladders below the vertebral column, and the same kind of structure was probably present in at least some Rhipidistia (markings on centra—Gregory & Raven, 1941; Westoll, unpubl.) and is of course characteristic of Dipnoi. It is probable, though not certain, that this body fulfilled a respiratory function in the fossil forms. Some such organ, together with a device such as the choanal passage, would be essential for the successful evolution of animals able to leave the water. It is noteworthy that air-bladders occur in actinopterygians, and possibly also in *Bothriolepis*, an antiarch.

(7) *Vertebral column*

Only meagre details are known in early Crossopterygii and Dipnoi. Watson & Day (1916) briefly described an imperfect caudal region of *Glyptolepis paucidens*, which they claimed was temnospondylous. Gregory, Rockwell & Evans (1939) and Gregory & Raven (1941) have described the structure in *Eusthenopteron foordi*, and Westoll (unpubl.) has observed somewhat similar conditions in *Osteolepis* and *Thursius*. Among later osteolepids the ring-vertebrae of *Megalichthys*, *Strepsodus*, etc. are familiar.

The 'central' elements of *Eusthenopteron* (Fig. 9 A, B) are paired, and adjacent elements are not in contact. They are half-rings applied to the large notochord, which may be slightly constricted. They correspond to the intercentra (=hypocentra) of Stegocephalia. Between them are small dorsal paired elements regarded as pleurocentra. The paired elements do not meet dorsally. In *Osteolepis* and *Thursius* the intercentra are united as split rings. In these forms many of the anterior (thoracic) intercentra have smooth flattened ventral surfaces, presumably due to contact with the air-bladder. The neural arches and spines are paired in *Eusthenopteron*, and tend to clasp those in front by rudimentary zygapophysis-like processes (Gregory & Raven, 1941). Slight regional differentiation is present. Haemal arches are borne by the posterior intercentra. There are some thirty-six vertebrae in front of the first haemal arch, and the pelvic fins originate at about the same place.

In *Megalichthys* and *Strepsodus* (cf. Gregory, Rockwell & Evans, 1939) the intercentra are complete biconcave perforated discs, and no remains of pleurocentra have been recorded; in the caudal region each ring-vertebra bears neural and haemal arches. Watson & Day's account of *Glyptolepis* would apply to many specimens of *Eusthenopteron* which suffered post-mortem displacement; their specimen needs to be re-examined.

No ossified ribs are known in osteolepids or holoptychiids. The coelacanths (Moy-Thomas & Westoll,

1935) and Dipnoi have pleural ribs, while tetrapods have dorsal ribs—a surprising difference (cf. Goodrich, 1930, p. 75). It is therefore interesting that the thoracic vertebrae of *Strepsodus* (Fig. 9 C) have often a paired process (presumably parapophysis), perhaps for the capitulum of a bicipital (?) rib, and Gregory *et al.* note somewhat doubtful parapophyseal areas on the intercentra of *Eusthenopteron*.

The earliest known tetrapod vertebrae are those of an undetermined 'lepospondyl' from the upper Dinantian of Scotland, and the very slightly later 'adelospondyls' from the same region, described by Watson (1926*b*). The persistent neurocentral suture is not a reliable character for ordinal classification

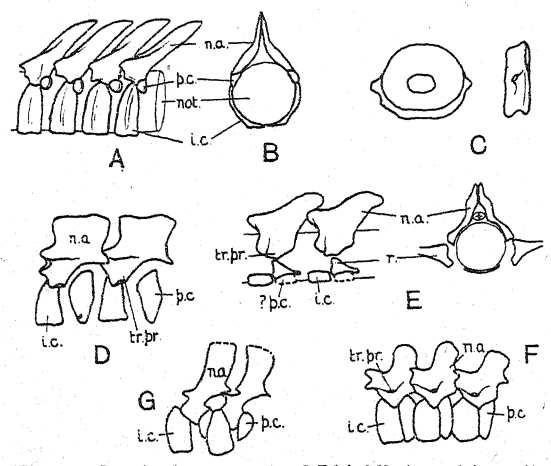

Fig. 9. Vertebral structures of Rhipidistia and juvenile Stegocephalia. A, B, Vertebral structure of *Eusthenopteron*, lateral and anterior views, after Gregory & Raven (1941). C, Ring-vertebra ('intercentrum') of *Strepsodus*, showing possible parapophysis, after Gregory & Raven (1941). D, *Stegops divaricata*, dorsal vertebrae, after Steen (1938). E, *Miobatrachus*, dorsal vertebrae and ribs, after Watson (1940). F, Young *Acanthostoma*, dorsal vertebrae, after Steen (1937). G, Young *Archegosaurus*, dorsal vertebrae, after Steen (1937).

Explanation of lettering: *i.c.* = intercentrum; *n.a.* = neural arch; *not.* = notochord; *p.c.* = (pleuro)centrum; *r.* = rib; *tr.pr.* = transverse process.

(cf. Steen, 1938, p. 272) and indeed Watson's figure of the 'lepospondyl' vertebra (1926*b*, fig. 23) corresponds extremely closely to Romer's figure (1930, fig. 12) of the vertebrae of the 'adelospondyl' *Pleuroptyx* (Steen, 1931). These vertebrae have each a single large centrum, the intercentrum being very small if present at all. The earliest known embolomerous forms are somewhat later. It is commonly accepted that the embolomerous condition is primitive in early tetrapods. This may be so in the true labyrinthodonts and anthracosaurs, but not necessarily in 'Microsauria' and perhaps not in the evolutionary lines leading to captorhinomorphs and diadectomorphs.

The embolomerous structure consists of approximately equal disc-like centra (pleurocentra) and intercentra (hypocentra), both supporting the neural arch; bicipital ribs attach to the transverse process of the neural arch and to parapophyseal facets borne essentially by the intercentrum. Juvenile stages of Embolomeri showing the development of the centra are not known, but young forms of late Carboniferous and early Permian Stegocephalia are most instructive (Fig. 9). Steen (1937, 1938), Romer (1939) and Watson (1940) have discussed new evidence that many of these forms were 'phyllospondylous' when young, i.e. had neural arches but no centra. Young *Archegosaurus* (Steen, 1937; Romer, 1939), after passing through such a stage, develop large intercentra and smaller paired dorsal (pleuro)centra (Fig. 9 G). *Acanthostoma* (Steen, 1937) shows paired intercentra and (pleuro)centra, as thin hemicylindrical shells, at one stage (Fig. 9 F), and *Stegops* (Steen, 1938) (Fig. 9 D) and some related types are rather similar. In *Miobatrachus* (Watson, 1940) gutter-like intercentra develop below the notochord, and more dubious remains of thoracic (pleuro)centra are reported (Fig. 9 E); the caudal region was probably 'rhachitomous', with dorsal (pleuro)centra and ventral intercentra.

These conditions in immature aquatic individuals are strikingly reminiscent of those in *Eusthenopteron*. The structure of the vertebral column in adult early tetrapods shows presumably functional adaptations correlated with the walking habit; e.g. the cantilever suspension of the weight of the body between piers (the legs) is clearly correlated with the close packing and well-developed zygapophyses of the vertebral elements.

The precaudal vertebrae of *Eusthenopteron* number about thirty-six, those of *Megalichthys* were perhaps slightly fewer. Early Stegocephalia have from about twenty-two to about thirty presacral vertebrae, and the first haemal arch is usually a few (about 3–6) vertebrae farther back. There was therefore probably no excessive multiplication or reduction in the number of precaudal vertebrae in the transition from osteolepid fishes to primitive tetrapods.

(8) *Paired limbs*

The older comparisons between the tetrapod limb and the paired fins of sharks and of *Polypterus* are now only of historical interest. There remains a wide range of opinion concerning the actual homology of parts of the tetrapod limb with the elements comprising the fin-skeleton of Rhipidistia or Dipnoi. Recent works of importance include those of Gregory (1935), Gregory & Raven (1941), Schaeffer (1941) and Holmgren (1933, 1939), and I hope to publish in the immediate future a review of this and other work in an account of the origin of the primitive tetrapod limb, the conclusions of which have been available in writing this article (see Summary, sect. 11). The paper in question contains the essential references. In brief, the conclusions of that work are that the tetrapod limb consists of (*a*) new structures

(neopodium) not present in the rhipidistian paddle, comprising the digits (phalanges plus the corresponding podialia), the prepollex or prehallux and (questionably) the postminimals being reckoned as digits; and (*b*) structures derived from the rhipidistian paddle with little modification (archepodium). The 'metapterygial axis' of Rhipidistia is represented in the humerus (femur)—ulna (fibula)—intermedium —two centralia, and is directed between digits I and II. These conclusions somewhat resemble those of Gregory & Raven (1941). It is also probable that all tetrapods are derived from ancestral forms with the same number (5 or 6) of mesomeres in the main axis. This suggests a closely monophyletic origin for all tetrapods.

The relationship between the skeleton of the paired fins of rhipidistians, coelacanths and dipnoans is also discussed in the paper mentioned above.

Changes in limb-girdles in the transition from fish to tetrapod have been discussed by Watson (1926*a*, etc.) and by Gregory & Raven (1941). In both pectorals and pelvics the assumption of a walking habit is associated with great development of endoskeletal limb-girdles. The necessity for upgrowth of the pelvic girdle and its firm attachment to the vertebral column is clear, otherwise important structures would suffer crushing. Watson (1926*a*) has shown that the pelvis was attached to the vertebral column only by ligaments and muscles in certain embolomeres; the firm articulation apparently came later. The shoulder-girdle also changes function; the dermal girdle is important in fishes, forming the posterior wall of the gill-chamber and the anterior attachment for the propulsive myotomic musculature. With the development of the neck (p. 94) and the new necessity for resisting strong upward thrust, the endoskeleton has developed in tetrapods a strong scapular blade which is firmly bound to the anterior thoracic ribs, and the dermal skeleton is gradually reduced. The development of the triradiate pelvis (and in principle of the compound endoskeletal shoulder-girdle) is discussed by Gregory & Raven (1941), who consider that the process is dependent upon muscular stresses.

The late Devonian (Chemung) tracks described as *Paramphibius* Willard are now known to have been made by invertebrates (Willard, 1935; Caster, 1938).

(9) *Body and scales*

Some part of the rhipidistian squamation is represented by the thin scales of many of the smaller Palaeozoic Amphibia, and by the thicker scales of some of the larger Stegocephalia and early reptiles. The same morphogenetic capacity is retained, as is well known, in many living types of Amniota. No tetrapod retains the cosmine-covered scale (*Kotlassia* is now known to have no cosmine tissue—Bystrow, 1940).

The differentiated median fins (anal, caudal, dorsals) of Rhipidistia are not present as such in primitive tetrapods. Primitive tetrapods probably showed a rapid 'degeneration' to continuous finfolds (i.e. loss of local differentiation), resulting in newt-like shape.

III. SIGNIFICANCE OF CHANGE FROM FISH TO TETRAPOD

(1) *Dynamic aspect*

It is reasonable to believe that the transition from fish to tetrapod did not happen as a sudden large-scale 'mutation'; the proto-tetrapod crossopterygians certainly showed traits and characters in structure and behaviour which were to be developed and emphasized in tetrapods, and conversely the early tetrapods must have resembled fishes in many ways. The question of locomotion must be viewed from this aspect, for it is likely that the immediate tetrapod ancestors used their paired fins in much the same way as the early tetrapods used their legs; and while it is probable that the crossopterygian might have been able to make its way over short stretches of mud-flats or sand-banks, it seems equally probable that the early tetrapods spent a large part of their lives in water (cf. evidence of latero-sensory canals). The tetrapod structure shows in several ways a marked advantage over the crossopterygian structure, but this would only be significant in shallow water or out of the water. Some features found in tetrapod limbs, for example their different normal carriage, increase in length of the proximal skeletal elements, torsion of the main axis (as compared with the Rhipidistian type), are easily understood as modifications of a pre-existing pattern dependent mainly on positional changes; they probably existed in almost entirely aquatic types. Others, for instance the great development of processes in the humerus for muscle-attachments and the new development of digital elements, may be regarded as secondary effects produced by new morphogenetic conditions resulting from the adoption of a new method of progression, involving the bearing of an important load by the distal part of the fin-axis; these changes were probably not made until the evolving proto-tetrapod stock was rather frequently using its paired limbs as legs where they had an appreciable load to bear, i.e. when out of the water, or in very shallow swampy conditions where the effect of buoyancy is reduced.

In the latter category come also the modification of the limb-girdles and vertebrae, modifications of the occiput, and possibly a whole series of skull-changes. The normal use during swimming of the paired fins of fishes imposes on their webs somewhat variable forces which are largely in the horizontal plane; positive or negative lift-forces may also be applied in favourable conditions, but these are probably not usually so large as the others. The rhipidistian dermal shoulder-girdle, to which the small scapula was rigidly fixed internally, was attached

anteriorly to the back of the skull, and the two halves were rather firmly united; the pelvic plates were probably firmly embedded in body-muscles, and were probably attached to one another quite strongly. The paired fins could be abducted and retracted with little trouble by muscles attached to the primary girdles, anteriorly and more or less medially to the articular surfaces. The increasing ability of the animal to move out of water imposed a new and considerable upward force on the girdles which if not countered would cause pressure on the heart and posterior abdomen. In the shoulder-girdle this was met by reinforcing the old supra-cleithrum to cranium attachment (later completely lost) by upward growth of the scapular blade so that it became firmly embedded in muscle; in the pelvic girdle by the upgrowth of a strong process (ilium) which was at first similarly fixed by muscular attachment to ribs and vertebral column, later by closer union with sacral ribs. The great muscles necessary for the clumsy laterally directed limbs would require increased areas of attachment on the girdles, which increase in size; and the markedly more anterior swing of the proximal element of the limb, together with the fact that the backward swing is the load-bearing propulsive movement, is sufficient explanation of the necessity for development of postglenoid or postacetabular surfaces for the attachment of the muscles concerned.

The vertebral column, becoming more fully ossified and linked by strong yet flexible union, and by the increasing development of serial apophyses for muscle-attachments, is clearly developing, as Gregory (following d'Arcy Thompson) has often emphasized, into a cantilever structure supporting the weight of the body, and it is understandable that detailed comparison of vertebral structure between rhipidistian and tetrapod is not easy.

Finally, it may be emphasized that habitual, even if temporary, emergence from the water (implying the loss of the buoyancy-factor) would be expected to result in considerable changes in the skull. The occiput would become the attachments of much more powerful muscles for turning and holding up the head (broad occipital plate, wide paroccipital processes), a mechanically sound system of neck-joints would have to be developed (atlas and axis, condyle), and the masticatory musculature would need to be strengthened to take the additional load of the weight of the mandible. A proper evaluation of some of these mechanical necessities would go far to explain the changes in skull-proportions.

(2) *Physiological aspect*

The transition from dominantly water-dwelling habitat to free movement out of the water was marked by certain remarkable changes in physiology of respiration, of excretion, of osmo-regulation and water loss, and of the special senses.

With regard to respiration, it is highly probable that Rhipidistia had well-developed air-bladders, since these are present not only in their coelacanth descendants and (as lungs) in tetrapods, but also in living Dipnoi whose Devonian ancestors were derived from the same stock as crossopterygians. With increasing freedom from the water the air-bladders must have developed into the main respiratory organ, and the arrangement of the nares would allow breathing with a closed mouth. Powers (1932) has shown that there are certain physiological difficulties in the simultaneous use of air-bladder and gills for respiration in teleost fishes; it is uncertain how far these considerations apply to the protetrapods. The loss of the entire bony opercular system in all known tetrapods may be significant of geologically early obliteration in the adult of the gill-clefts; but many juvenile Stegocephalia (including phyllospondyls) had long external gills, and Bystrow (1938*a*) has discussed *Dvinosaurus* as a neotenic form in this respect.

Osmo-regulation cannot profitably be discussed here. It seems probable, however, that the nature of the skin of Rhipidistia, with its heavy ossification, was such that it gave considerable protection against osmotic difficulties, and a similar skin would be expected to be of service with little modification as a protection against desiccation.

The optics of vision during the period of transformation has long been a difficulty, but this has been largely overcome by the discovery of Hogben & Landgrebe (1940) that the eye in some fishes is capable of forming an image on or near the retina either in water or in air. In water the cornea, vitreous humour and outer zones of the lens (all with refractive index about 1·35) are not active optical agents, but the lens has a sharply marked core of refractive index about 1·5, which forms an image on the retina. In air the convex cornea-air interface is the main image-forming optical structure. Fish eyes tend to be almost hemispherical; the almost spherical eyeball of higher vertebrates was presumably a rather slowly evolved character specially adapted for vision in air.

K. von Frisch and his school have investigated in some detail the sense of hearing in living fishes (von Frisch, 1936,* 1938*a*, 1938*b*). From this it appears that in Ostariophysi (in which the swim-bladder and Weberian ossicles play a great part in sound perception) sound-stimuli are perceived over a considerable range, from very low frequencies up to about the same high frequency as can be perceived by the human ear. The high-frequency perception is apparently through the sacculus and lagena. Low-frequency vibrations are said to be perceived 'by the touch sense only'; von Frisch has apparently not considered in detail the role of the latero-sensory system. In non-Ostariophysi the high-frequency perception is apparently less acute. It seems likely that the acuity of hearing in Ostariophysi is a special feature correlated with the development of the

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Weberian ossicles and modified swim-bladder, which show an extraordinary parallelism to the auditory ossicles and tympanic cavity of mammals. Perception of low-frequency vibratory stimuli in fishes is therefore largely through the medium of the neuromasts of the latero-sensory system and apparently far less through the internal ear. The internal ear, though originating from a placode comparable in some ways with the latero-sensory system, and innervated by fibres leading to the same acustico-lateralis tract, mainly records changes in the gravitational field affecting the animal (semicircular canals and sacculus). With the loss of the real effectiveness of the latero-sensory neuromasts in most tetrapods the whole 'auditory' function has been taken over by the inner ear, and through the tetrapods is an interesting development of structures (lagena → cochlea) for the increasingly efficient reception of sound-waves. The modification of the hyomandibular into a functional columella auris (stapes) has been analysed elsewhere, together with the development of a tympanic cavity and membrane. It seems probable that the 'columella auris' and the tympanic cavity were developed before the latero-sensory canals were fully degenerated, and the perforate fenestra ovalis apparently was a later development.

The retention of the mechanism of olfactory perception would necessitate the development of mucous membranes lining the olfactory chamber, capable of being kept moist at all times.

(3) *Evolutionary mechanisms and speed of change*

It is usual to assume that animals so different in detail (but structurally allied) as Rhipidistia and tetrapods, or Rhipidistia and Dipnoi, or various groups of early Amphibia, or (to take a later example) the therapsid reptiles, must in each case have evolved from some common stock, the evolutionary change taking place slowly over many millions of years. Thus Säve-Söderbergh (1933) suggests that the common 'choanate' ancestor was of early Devonian or Silurian age, and Steen (1938, fig. 47) shows the main groups of Amphibia and Reptilia as separate in the Upper Devonian and converging only in much earlier times. I believe this to be an erroneous view, for which there is not the slightest palaeontological evidence in any case; my reasons are threefold—the absence of palaeontological evidence of long divergence, certain factors of development of structures in ontogeny, and some reflections on the nature of structural change.

The tetrapods differ from the Rhipidistia in a number of ways which (as shown above) are related to changes of environment and of locomotory habits. If the process of differentiation had been prolonged the intermediate stages would have lost much of their efficiency as fishes, while remaining comparatively inept out of the water. I consider that these stages were passed through quickly, and that *Elpistostege* from the lower part of the Upper Devonian is a true proto-tetrapod not long diverged from normal Rhipidistia. Between this time and the late Upper Devonian the primitive tetrapods had not only become well established, but had already proceeded along several divergent lines of evolution. It is not impossible that a comparatively short period of very unfavourable conditions (shallow swamps, etc.) saw the extinction of some less adaptable types, and the initiation in the fish ancestors of the tetrapods of new adaptations to a life in which buoyancy may have played only a small part in the support of the body and its component parts; the whole process may have taken place in a single geographical region.

Modern embryological investigations have shown convincingly that the development of many structures in vertebrates depends on the regular appearance and functioning of centres of organization. Each such 'centre' determines and directs a specific differentiation of tissues which until that time were capable, under appropriate stimulus, of a variety of reactions. Once 'organized' immediate differentiation proceeds even in rather adverse conditions, but sooner or later new organizer-reactions normally affect parts of the structures formed in the meantime and carry the process further. Moreover, single structures and organs may fall within the field of influence of different organizers. It has occurred to me that such considerations may give a clue to the apparently rather sudden appearance of new types. Imagine a succession of individuals gradually becoming genetically adapted to a change of function and environment. Minor changes of proportion and shape of structures will only necessitate a change in the ontogenetically late members of the organizer-chains, the main pattern being still of the same type. In these changes the modified organizers will produce a different pattern of differentiation in the labile tissues they affect. More profound genetical changes during the adaptation may affect earlier determined organizer-reactions and may thus, abruptly, greatly modify all subsequent differentiation, submitting the labile tissue to the predominating action of other organizers and producing a structural pattern of perhaps markedly different character. Such changes may be frequent, but viable products must be rare. Whether this suggestion be true or not, it is clear from genetical work that even single-gene mutations may so modify normal developmental patterns as to produce great 'plastic' changes and highly modified structures and patterns. These single-gene effects are, of course, far too crude an explanation of the emergence of new vertebrate types, but they do at least show the effect of genetic change, presumably through the disturbance of normal organizer chains, on macroscopic pattern, and show some of the possibilities of genetic control of altered relative growth, a phenomenon of wide application in vertebrates.

IV. MONOPHYLETIC ORIGIN OF TETRAPODS

The most primitive Reptilia are certainly derived from immediate ancestors which, if not actually known types of early 'Amphibia', must have been referable to that group, and have shared with them a common plan of structure of every part of the skeleton. The Amphibia have been supposed by several recent authors to be at least diphyletic, urodeles being derived from a different group of fishes (usually Dipnoi) from those Rhipidistia believed to be ancestral to the other tetrapods. This supposition has been based on several factors, for example, the structure of the palate (Wintrebert) and of the limbs (Holmgren), details of histology (Kerr), and some work on the development of the excretory organs (Kindahl). The structure of the limbs is actually, as shown above, a strong factor in favour of the descent of urodeles and other tetrapods from a common ancestral *tetrapod* plan: the difference in palatal structure is worthless when the palaeontology is considered: and the other points are partly explicable by remote ancestral community, and partly by the occurrence of similar physiological and structural mechanisms in fishes and amphibia living in somewhat similar environmental conditions.

The facts and arguments set out above show beyond any doubt that the primitive tetrapod was derived from a generalized rhipidistian crossopterygian. Even the most conservative analysis shows that the Dipnoi are in no way implicated in the immediate tetrapod ancestry, but it is possible to go much further. The proto-tetrapod type was already well differentiated in earliest late Devonian times (though still showing proportional resemblances rather to Rhipidistia than to Stegocephalia), and the process was probably already completed during the late Devonian, because there is already a considerable radiation of amphibian types in the early Carboniferous. Furthermore, there is extremely strong evidence that all the early tetrapods were derived from a very narrowly defined group of Rhipidistia. The reasons for this statement are cumulative. It would no doubt be possible for parallel evolution of rather similar but not identical forms to result in somewhat similar changes in, for instance, skull-patterns and proportions, or development of the nasal capsule and its surrounding dermal bones, or production of a backwardly extended parasphenoid, or modification of hyomandibular into a columella auris, or modification of a rhipidistian paddle into a walking limb, or increased development of endoskeletal shoulder and pelvic girdles; but it is entirely unreasonable to suppose that these and other changes could have happened cumulatively and independently with results so very similar in minor details. To take only the limbs as an example (to be discussed elsewhere), it seems highly probable that the rhipidistian paddle suitable as the ancestral type for *all* tetrapods had five well-developed metapterygial mesomeres each with a preaxial radial, and the rudiment of a sixth mesomere: that each radial was a single rod: that the pectoral and pelvic fins were similar in structure: and perhaps that the modifications of the fin for walking involved the separation of the actual or potential postaxial processes of the second and subsequent mesomeres, and the development of new structures (digits) from postaxial mesoderm. Such a series of processes could not produce identical results except from stocks with identical meristic and mechanical structure of the paddle. Similar conclusions may be drawn from the study of skull-pattern and other characters. I do not think it an exaggeration to suggest that the true proto-tetrapod type evolved once and once only, perhaps from a single species, almost certainly from a single genus; and it is very likely that this evolution took place in the area of deposition of the late Middle and early Upper Old Red Sandstone rocks including the present remnants in E. Canda, E. Greenland, Spitzbergen, W. Norway and Britain, more probably in the present western parts of the area. I hope to present the stratigraphical and palaeogeographic evidence on another occasion.

V. SUMMARY

1. Only Crossopterygii* and Dipnoi can be considered as possible tetrapod ancestors. Early tetrapods converge on Stegocephalia and allied groups in early Carboniferous time. These and the Devonian fishes are therefore the key-material.

2. The skull structure of all tetrapods is based on a common plan which can only be derived from osteolepid Rhipidistia. The Dipnoan skull is divergently modified, and the holoptychiid and coelacanthid Crossopterygii are too specialized for consideration. Various attempts to derive some tetrapods (urodeles) from Dipnoi are untenable.

3. Analysis of the skull structure shows that great changes in proportion took place during the evolution of a primitive tetrapod from an osteolepid structural pattern. There is also considerable modification of the dermal bone pattern of the anterior skull roof. This has led to erroneous homologies. The 'orthodox' homologies, and those of Säve-Söderbergh and Allis, are incorrect and misleading. A straightforward bone-to-bone homology between osteolepids and tetrapods is probable, except for the frontals and nasals of the latter, which represent concentrations from the long series of osteolepid 'nasals'. The tetrapod parietal is homologous with the element usually called 'frontal' in osteolepid and other fishes. The differential fusion of elements postulated by Säve-Söderbergh has no basis in fact.

4. The kinetic skull of osteolepid Rhipidistia is no bar to the belief that they include the tetrapod ancestors.

5. The teeth of early tetrapods and of Rhipidistia are based on similar patterns.

6. The histology of the dermal bones of Stegocephalia is similar to that of large Rhipidistia, but the cosmine layer is lost in the former.

7. The morphogenetic significance of the latero-sensory system in relationship to dermal bone formation is discussed. It is apparently of great value where it is

* *Polypterus* is an actinopterygian.

developed as enclosed canals, but pit-line grooves and superficial sulci are of little importance, as shown by the migration of the sulci over the dermal skull of Stegocephalia.

8. New work throws much light on the hyomandibular-stapes problem and the early evolution of the tetrapod ear and tympanic cavity.

9. The phylogenetic modification of the external nostrils in osteolepids and Stegocephalia is remarkably similar to the ontogenetic development of the face even in mammals. There may be two bones confused under the name 'septomaxillary' in tetrapods.

10. The vertebral column and ribs are considered. Many features of adult early tetrapods are explicable on mechanical grounds as correlated with change of habitat and loss of aquatic buoyancy. The problem presented by the pleural ribs of coelacanths and Dipnoi is indicated.

11. Recent work on the origin of the tetrapod limb (to be reviewed and discussed in a separate publication) is briefly summarized. It seems probable that the digits and their carpals or tarsals are new developments (neopodium), the remainder of the cheiropterygium (archepodium) being derived with comparatively small change from the rhipidistian paddle. The metapterygial axis is represented in tetrapods by humerus (femur)—ulna (fibula)—intermedium—two centrals, and is directed between the podials of digits I and II. Both urodeles and other tetrapods show the same basic pattern.

12. The early radiation of tetrapods is analysed, and it is shown that there is no good evidence against a monophyletic origin of a single basal type from which all others were derived.

13. The dynamic significance of the emergence of tetrapods lies largely in locomotion mechanics and the loss of the buoyancy effect on leaving the water. The effects on limbs, skull, neck region and vertebral column are considered.

14. The physiological changes in respiration, excretion and 'special senses' of hearing, vision, etc. are briefly noted.

15. The process of emergency of tetrapods was probably comparatively quick, incipient in the earliest late Devonian and completed before the early Carboniferous.

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ADDENDA

To §(4), p. 89, add: Holmgren & Stensiö (1936, p. 351) suggest that the primitive coelacanth '*Diplocercides*' (Nesides) had an incipient fenestra ovalis. Romer (1937, p. 39) shows that this is improbable as a result of his examination of *Ectosteorhachis*, in which, however, there may have been a rudimentary fenestra rotunda. Watson (1926*a*) showed that some Embolomeri had an unperforated fenestra (pseudofenestra) ovalis.

Two recent works, by Sawin and by Romer & Witter, have added much to our knowledge of primitive Stegocephalia. The cranium described by Romer and Witter shows far-reaching resemblances to that of osteolepids, and would give a better 'fit' than fig. 5*c* of this review.

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CAMBRO-ORDOVICIAN CEPHALOPODS

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I. INTRODUCTION

It is now known that authentic cephalopods were widespread and locally abundant as early as Upper Cambrian and Lower Ordovician times. The preservation of most of the specimens leaves much to be desired, but the large collections that have recently been studied reveal a surprising amount of information. Most of the material consists only of silicified internal moulds, but some specimens preserve the details of the surface markings of the test, others the early growth stages, and still others the internal structures. Specimens which happen not to have been silicified are particularly helpful, for they can be sectioned. Also, it should be kept in mind that internal moulds of cephalopods are as a general rule more satisfactory to study than are those of related invertebrates, and from a careful examination of the wealth of material in the various American museums certain generalizations have become apparent.

II. CHRONOLOGICAL DEVELOPMENT

The oldest fossils that are commonly regarded as cephalopods are the Lower Cambrian genera *Volborthella* and *Salterella*, which are at least superficially similar. The affinities of these forms have always been a moot question. Several competent palaeontologists have contended that they are orthochoanitic nautiloids, whereas others are of the opinion that they should be regarded as pteropods, or possibly conularids or Foraminifera. Within recent times their status has been reviewed at some length by Teichert (1929), Schindewolf (1928, 1929, 1934*a*, *b*, *c*), and Spath (1933, 1936, 1937), as well as by myself, but there is little unanimity of opinion. In 1933 the outstanding American student of nautiloids, Foerste (together with Ulrich), accepted *Volborthella* as a cephalopod, but before his death some three years later he had become very uncertain in regard to its relationship. Although I do not consider the evidence

unequivocal, none of the numerous specimens I have studied, both externally and in thin section, has convinced me of the cephalopod affinities of these forms. Furthermore, it has always amazed me that anyone can look at a slab of Estonian blue shale containing topotypes of *Volborthella tenuis* (the only described species of the genus) and see any reason to doubt that this form is a pteropod. Finally, it should be emphasized that between the horizon of *Volborthella* and that of the oldest undoubted cephalopods there is a considerable interval from which not even a trace of intermediate types is known.

When such problematical forms as *Volborthella* and *Salterella* are excluded, the oldest known cephalopods seem to be two species of *Plectronoceras* from the Upper Cambrian of north-eastern China and Manchuria (Fig. 1). This genus includes simple little slightly curved brevicones with elliptical cross-section, essentially smooth test, nearly straight

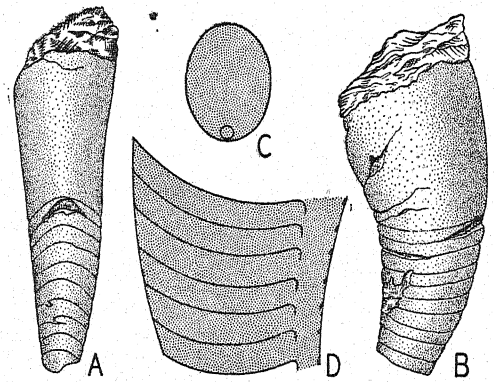

Fig. 1. Three paratypes of *Plectronoceras cambria* (Walcott) from the *Tsinania* zone in the Upper Cambrian Ch-au-mi-tién limestone west of Tsi-nan, Shantung, China. A and B, which represent one specimen, are ×5; C is ×5; and D is ×12½.

sutures, and marginal siphuncle located next to the concave side of the conch. Kobayashi (1935) states that in the Manchurian species the siphuncle is cyrtochoanitic in structure; that is, that the septal necks are recurved and the connecting rings are greatly expanded within the camerae, and this conclusion was accepted by Foerste. It should be noted, however, that only one connecting ring was observed, and I am inclined to doubt its authenticity, though I have not seen the specimen (longitudinal section) on which the observation is based. Furthermore, in the type specimens of the genotype, which came from north-eastern China, the septal necks are essentially straight, and they are almost half as long as the camerae (Fig. 1 D). Connecting rings are not preserved in any of the specimens sectioned, but I think it most probable that they are cylindrical as are those of other Early Palaeozoic nautiloids. None of the numerous forms known from the Cambrian and Lower Ordovician of America has the siphuncular segments appreciably expanded within the camerae, but early Middle Ordovician (Chazyan) cyrtochoanites are quite varied, suggesting a fairly early origin for the group.

In America cephalopods made their appearance in the uppermost Cambrian Potosi and Eminence formations of the Ozark region. The few specimens that have been found in these formations are placed in the genus *Shelbyoceras*, though the nature and position of their siphuncle is not known. In so far as general physiognomy and sutures are concerned, they resemble *Plectronoceras* rather closely, but they are considerably larger, are more rapidly expanded orad, and are oval in cross-section, being almost subangular along their convex side. Specimens that may be conspecific with the Potosi form are known also from central Alabama, and it should be mentioned that the uppermost Cambrian of south-western Oklahoma has yielded a few poorly preserved fragments that appear to be orthoceraconic cephalopods. In the Ozark region brevicones are locally abundant in the basal Ordovician Van Buren formation, and the overlying Gasconade dolomite has yielded a large and varied molluscan fauna with many types of breviconic and longiconic nautiloids. This latter fauna is widespread in America, being particularly well represented in the Upper Mississippi Valley and in the Southern Appalachian Highlands, and according to a personal communication from Prof. F. W. Whitehouse it is well developed in north-eastern Australia. Furthermore, small collections seem to indicate that the same (or a closely related) fauna is probably represented also in various parts of Canada, western Alaska, north-western Greenland, and eastern Asia.

Following this period of abundance during earliest Ordovician times there seems to have been a material decline in the ranks of the cephalopods. However, during the closing stages of the Lower (pre-Chazyan) Ordovician there was another great advance which carried the group far beyond the goals that it had achieved before. This second climax is characterized by the appearance of the piloceratids and particularly by the presence of the first coiled nautiloids, which, to be sure, occur in association with a variety of breviconic and longiconic types. As might be expected, this development at the close of the Lower Ordovician was more or less world-wide in its effects. The best preserved and most diversified faunas that have so far been recorded are from the Lake Champlain region of America, but cephalopods of this age are not rare at many localities in the United States, Canada, Newfoundland, and north-western Scotland, and they occur also in northern China, Manchuria, Korea, Bear Island, and probably Norway and Alaska. They can be said to foreshadow the great development of the nautiloids during the rest of the Ordovician and the following two periods of the Palaeozoic.

III. SHELL MORPHOLOGY

The majority of the Cambro-Ordovician cephalopods are brevicones. However, straight and curved longicones are fairly abundant, and at a few localities Lower Ordovician nautilicones are not particularly rare. Most of the brevicones and a considerable proportion of the longicones are curved. In many of them the curvature is endogastric, whereas in a few others it is exogastric. Shells that are compressed laterally are more numerous than those that are circular in cross-section or those that are depressed dorso-ventrally.

In most of the Lower Ordovician nautilicones the volutions are depressed during early adolescence, but they show a distinct tendency to become relatively narrower during later growth stages. The impressed zone, where present, becomes relatively deeper during ontogenetic development. In closely coiled forms like *Trocholitoceras* an impressed zone is developed before the whorls are in contact. As full maturity is approached, in essentially all Lower Ordovician nautilicones the living-chamber diverges from the preceding whorl of the conch. In some forms at the point of divergence the impressed zone is lost and the dorsal side of the whorl is merely flattened. In others the depth of the impressed zone is diminished when the living-chamber diverges, but the dorsal zone appears to remain slightly concave throughout ontogenetic development. Only a few specimens are known that preserve the apertural margins, but in at least some of the coiled forms the aperture is considerably restricted dorsally when full maturity is attained.

Like other cephalopods, during late stages of growth, the Early Palaeozoic forms developed progressively shorter camerae, as well as modified apertures in some cases. Specimens in which the adoral septa are crowded and the aperture appreciably modified were regarded by Hyatt and Ruedemann as gerontic individuals. In coiled forms a divergent living-chamber was also regarded as evidence of senility. However, it now seems that these features are merely characteristic of full maturity. In most ammonoids and other nautiloids, as well as coiled gastropods, the development of comparable features is generally regarded as evidence of maturity. Presumably specializations of this type were made so that the animal would be better adapted to its environment, and they are not a response to senility.

The living-chamber of the Early Palaeozoic orthoceracones and cyrtoceracones is quite variable in both size and shape. However, the available data seem to indicate that all of the Lower Ordovician nautilicones have living-chambers that are uniformly about one-half of a volution in length, and at full maturity the portion of the conch occupied by them is more or less divergent. The significance of these facts is of course problematical, but they may indicate that at maturity there was a marked change in habits or habitat, or both. The alteration of the shape of the conch may have been merely a simple modification to facilitate mobility and may not indicate a relationship to such later forms as the Lituitidae, in which the phragmacone as well as the living-chamber became essentially straight.

Traces of the increments of growth can be discerned on many specimens, and some of those that retain the test (or a replacement of it) show the details of the surface markings. The growth lines vary considerably in prominence, and in a few types they are fasciculate and form distinct ribs. In the majority of forms the growth lines and the ribs, when present, curve apicad toward the venter and form hyponomic sinuses. Thin sections of the test of some of the Lower Ordovician nautilicones show that it is composed of two main layers. The outer one is relatively thin, and the growth lines are probably confined to it. A *Runzelschicht* or inner wrinkled layer has been observed in only one species, *Curtoceras internastriatum*. It resembles that of the younger nautiloids and ammonoids, and a similar structure is probably present in most or all forms but is more prominent in some than in others.

For the most part in these early nautiloids the sutures are merely an expression of the shape of the conch. Accordingly, lateral lobes are present in forms with compressed conchs, and coiled shells that are impressed dorsally have dorsal lobes. However, in some forms, for example in *Catoraphiceras*, there are prominent lobes due to inflections of the septa.

The siphuncle of the oldest undoubted cephalopods, *Plectronoceras*, is ellipochoanitic; that is, the septal necks are so short that they do not extend entirely from one septum to the next, and presumably the gaps between them are occupied by thin structures known as connecting rings. Kobayashi (1935) believed the siphuncle of *Plectronoceras* to be strongly cyrtochoanitic, but I think it is probably orthochoanitic; possibly both of us made correct observations and the forms that we studied are not congeneric, or even closely related. Cyrtochoanitic siphuncles are expanded within the camerae, whereas orthochoanitic ones are not; both are, however, ellipochoanitic. Some of the other Early Palaeozoic cephalopods have holochoanitic siphuncles. In these forms each septal neck is long enough to invaginate into the infundibular adoral end of the next neck apicad, and the siphuncle therefore consists entirely of septal necks. Unfortunately the poor preservation of many of these Early Palaeozoic forms makes it impossible to determine whether their siphuncles are orthochoanitic or holochoanitic. In general it can be said that small cylindrical siphuncles are orthochoanitic and large subcylindrical ones are holochoanitic, but there are exceptions to these generalizations. It should also be noted that in longitudinal sections holochoanitic siphuncles appear to be composed of segments that are slightly but distinctly concave exteriorly.

In all of the Lower Ordovician nautilicones the

siphuncle is orthochoanitic in structure, the septal necks being short and straight. The connecting rings are essentially cylindrical, but in a few cases they are slightly concave exteriorly. Typically there is a tendency for the siphuncle to be relatively large during both early adolescence and late growth stages. In at least some cases its relative diameter increases appreciably as full maturity is attained.

Only the siphuncle is known of most of the piloceratids. Longitudinal sections of limestone specimens of *Cassinoceras*, a typical piloceratid, seem to indicate clearly that the siphuncle is holochoanitic in structure. In this genus, and probably in all the rest of the piloceratids, the adapical end of the siphuncle of mature specimens is, in some cases at least, filled with closely invaginated endocones, through the perforate centres of which runs a narrow endosiphotube that bears a few transverse partitions (Fig. 2 A). It is generally assumed that these endocones served as ballast, but this explanation does not seem very satisfactory.

Most of the other Early Palaeozoic brevicones belong in the Cyclostomiceratidae and the Cyrtendoceratidae, in which the siphuncles are orthochoanitic in structure. In at least some representatives of the Cyclostomiceratidae there are thin calcareous deposits on the outside of the siphuncle, but no endocones or other calcareous structures were observed on the inside of the siphuncle in members of this family. In a few specimens that belong in the genus *Levisoceras* of the Cyrtendoceratidae there are transverse partitions or diaphragms in the adapical portion of the siphuncle. These are only moderately closely spaced and are more or less saucer-shaped, being convex apicad; apparently they are not perforate and are therefore not connected by an endosiphotube. Like endocones, which also occur in some members of the Cyrtendoceratidae, these diaphragms are cemented to the inside of the siphuncle and their spacing is independent of that of the septa. At present I am of the opinion that diaphragms and endocones are probably homologous, but at the same time I want to emphasize that these structures need to be studied in more and better-preserved material than is now available.

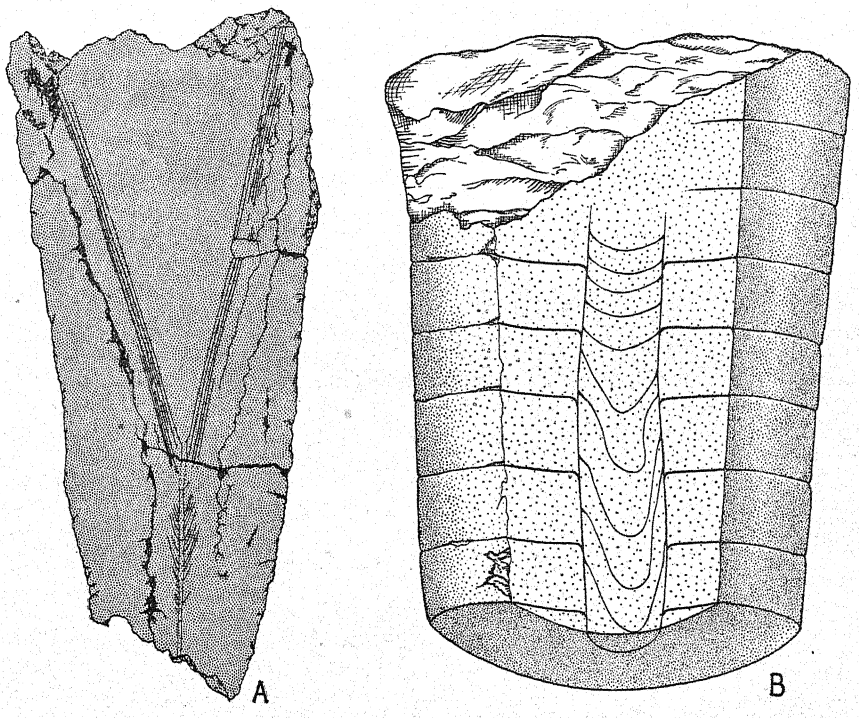

Fig. 2. Lateral longitudinal sections of (A) the holotype of *Cassinoceras amplum* (Dawson), a siphuncle from the late Lower Ordovician near Lachute, Quebec, × 1; and (B) a syntype of *Robsonoceras robsonense* (Walcott) from the early Lower Ordovician Cushina formation at Billings Butte, British Columbia, × 9.

In the longicones the siphuncles are quite variable. Some of them are definitely orthochoanitic and others are definitely holochoanitic. However, in the great majority of the specimens now available the structure of the siphuncle can only be inferred. The nature of the deposits inside the siphuncle, though admittedly very important, is difficult to determine because of the fact that most of the specimens are silicified. However, in well-preserved specimens of *Robsonoceras* (Fig. 2 B) there are deep subconical diaphragms in the adapical portion of the siphuncle, and these

become saucer-shaped in the adoral portion of the siphuncle of the same specimen. In at least some Lower Ordovician longicones, for example *Proterocameroceras brainerdi* of the Cassin limestone of Vermont and New York, there are closely invaginated endocones and an endosiphotube in the adapical portion of the siphuncle, just as in the piloceratids, and it seems reasonable to suppose that these exist in many more forms than in those in which they have been found.

One of the most peculiar of the Early Palaeozoic orthochoanites is *Buttsoceras adamsi* of the late Lower Ordovician of Alabama. In most respects this species is a typical orthoceracone with subcircular cross-section, essentially straight sutures, and rather small central siphuncle. However, in the centre of the siphuncle of well-preserved specimens there is a long

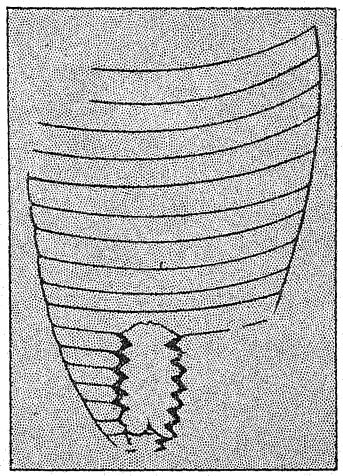

Fig. 3. Oblique section through the siphuncle of a unique nautiloid from the Lower Ordovician Rochdale limestone near Rochdale, New York, × 3.

slender tube which expands orad somewhat more rapidly than does the siphuncle. Several of the available specimens show this tube, so it is almost certainly not extraneous, but unfortunately the nature of its junction with the walls of the siphuncle is not known. Any similarity it may have to the endosiphotube of the holochoanites is, I believe, superficial, but I have no suggestion to offer in regard to its homology or function.

The Lower Ordovician Rochdale limestone of New York has yielded a unique form (Fig. 3) in which the walls of the siphuncular segments are concave exteriorly and in longitudinal section appear to be solid V-shaped structures with the apex of the V centrally located and pointed inwards. Our knowledge of this species is limited, for it is known from only a few oblique polished sections, and these do not reveal just which portions of the siphuncular walls are septal necks and which are connecting rings. Nevertheless, I am inclined to believe that the septal necks are short and that it is the connecting rings that are V-shaped in section.

Where it has been observed, the siphonal caecum is in contact with the apical end of the shell much as in modern *Nautilus* (Fig. 4). In the Trocholitidae the siphuncle is at first ventral in position, but it migrates to a dorsal position during the first volution of the conch, whereas in other Lower Ordovician nautilicones it remains ventrad of the centre throughout ontogenetic development or gradually assumes a subcentral position. During the ontogenetic development of *Trocholitoceras* the siphuncle becomes dorsal and marginal in position, but at full maturity it is again removed from the dorsal wall of the conch.

The umbilical perforation of Lower Ordovician nautilicones is invariably small and is comma-shaped

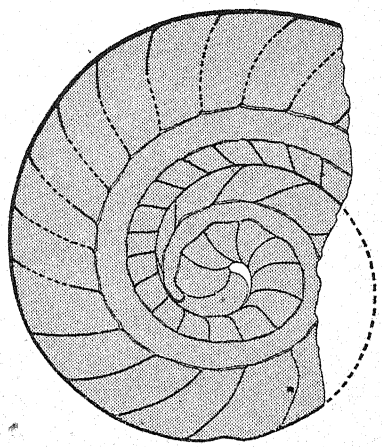

Fig. 4. Median longitudinal section of a syntype of *Curtoceras eatoni* (Whitfield) from the late Lower Ordovician Cassin limestone at Fort Cassin, Vermont, × 5.

in longitudinal section. In a few forms no perforation is discernible.

Most of the specimens available are so poorly preserved that the details of the inner volutions of the conch cannot be studied satisfactorily. However, in *Curtoceras* at least a constriction representing a nepionic line is present near the end of the first volution (Fig. 4). A nepionic line is prominent in ammonoids and occurs also in most nautiloids, including modern *Nautilus*. Willey (1897) has concluded that it indicates the size of the shell when the animal hatches from the egg, whereas Böhmers (1936) has suggested that it may mark the position of the aperture when the first septum is secreted. Both may be correct. In some forms the general appearance and especially the surface ornamentation of the test change markedly orad of this line; Hyatt (1900) used it to divide his nepionic and neanic stages of growth. Its position is constant within any given group, but varies considerably from one family to another. In

general, Devonian ammonoids form it at about the end of the first half-volution, whereas in later Palaeozoic ammonoids it is not formed until the conch has completed about a volution.

At least some of the Lower Ordovician nautilicones have lamellar deposits within the camerae, and these deposits appear to be primary (Fig. 5). They have only a certain amount of regularity, but are fairly symmetrical and extend over the ventral wall of the conch to the exclusion of the dorsal wall. There is a tendency for these deposits to be less strongly developed in the mature whorls. Such deposits are known in later forms, particularly in Middle and Late Palaeozoic longicones, and they appear to have been secreted by cameral tissues after the formation of the septa. Since they are relatively thin in the adoral portion of the phragmacone, it is generally believed that they served as ballast. Apparently they are not present in the holochoanites, but they are being found in many orthochoanites and cyrtochoanites of the Palaeozoic and the Triassic. A great deal remains to be learned about these deposits, and it is highly desirable that more and better criteria be developed to distinguish them from inorganic deposits, which they resemble closely. Structures have also been observed in the siphuncle of certain Lower Ordovician nautilicones that are believed to be primary deposits. As shown by Fig. 5A, these are more or less regular in their development. They take the form of a partial lining of the siphuncle and are developed particularly along the septal necks.

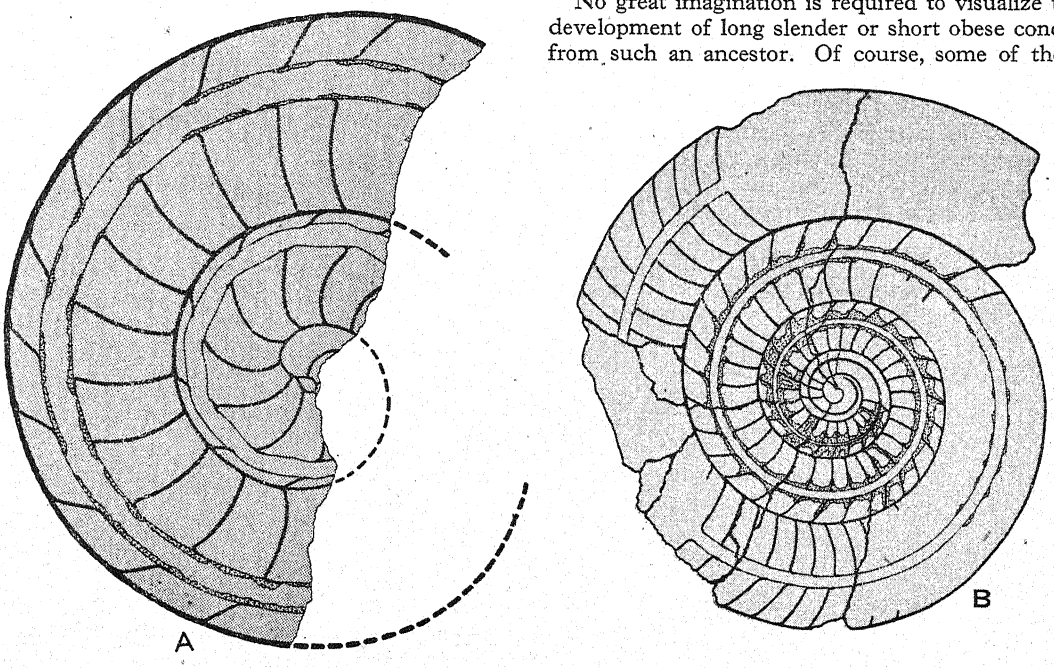

Fig. 5. Median longitudinal sections of two specimens of *Centrotarphyceras seelyi* (Whitfield) from the late Lower Ordovician Cassin limestone at Fort Cassin, Vermont. A × 5, B (based on a syntype) × $\frac{3}{4}$.

IV. EVOLUTION OF THE EARLY CEPHALOPODS

It is not safe to assume that our collections are sufficiently complete to show chronologically the details of the early evolution of the cephalopods. Nevertheless, it can be stated that the available data indicate that the first cephalopods were small curved brevicones with ellipochoanitic siphuncles, and with the exception of a few questionable forms all of the Upper Cambrian species seem to be of this type.

No great imagination is required to visualize the development of long slender or short obese conchs from such an ancestor. Of course, some of these descendants might be straight whereas others could just as readily be curved endogastrically or exogastrically; and some could be circular in cross-section whereas others could be compressed laterally or depressed dorso-ventrally. Gyroceracones have not been reported from the Cambrian, and only one poorly known species, *Beekmanoceras priscum*, has been found in the Lower Ordovician. Presumably this form developed from some of the earliest Ordovician cyrtoceracones, and it represents more or less of an intermediate stage between them and the nautilicones of the uppermost Lower Ordovician. Annulations are believed to have been evolved independently in several types of Early Palaeozoic nautiloids, probably by the growth lines becoming first prominent and then fasciculate.

The development of the various types of siphuncular structure is much more difficult to comprehend. Formerly it was believed that the earliest cephalopods

had holochoanitic siphuncles; that is, that each septal neck projected apicad far enough to invaginate into the infundibular adoral end of the next neck, so that the siphuncle consisted entirely of septal necks. It was then contended that as development proceeded, in certain forms there was a tendency for the necks to become shorter, and that the gaps that thereby arose between adjacent necks became closed with thin, more or less porous, structures called connecting rings. Such siphuncles, composed of alternating septal necks and connecting rings, are said to be ellipochoanitic. It now appears that we must reverse our ideas in regard to this evolution, for the oldest genera invariably have ellipochoanitic siphuncles, and Flower (1941, p. 1) has recently stated that 'the earlier holochoanitic forms, of Chazyan [basal Middle Ordovician] age, retain connecting rings, clearly a heritage from their ellipochoanitic ancestors'. However, I must admit that I have not been able to observe these vestigial connecting rings in any of the Lower Ordovician holochoanitic forms that I have studied. It appears that the holochoanites made their appearance in Lower Ordovician times, became very abundant during the Middle and Upper Ordovician, dwindled throughout the Silurian, and became extinct shortly thereafter. Apparently they represent a specialization that was quite successful for a time but was not enduring.

There are two types of ellipochoanitic siphuncles, orthochoanitic and cyrtochoanitic. In the former the septal necks are straight, the connecting rings are cylindrical, and the segments of the siphuncle are not expanded within the camerae; whereas in the latter the septal necks are recurved and the connecting rings are fusiform, subspherical, or even nummuloidal in shape. The majority of Cambrian and Lower Ordovician cephalopods had orthochoanitic siphuncles, and it seems likely that true cyrtochoanitic types are not known to exist before the Middle Ordovician (Chazyan). Presumably they developed from the orthochoanites as did the holochoanites.

V. SUMMARY

Volborthella and *Salterella* of the Lower Cambrian are probably pteropods, rather than cephalopods. The oldest undoubted cephalopods are two species of *Plectronoceras* from the Upper Cambrian of eastern Asia; both are small curved brevicones with ellipochoanitic siphuncles. The oldest American cephalopods are from the uppermost Cambrian of the Ozark region, and they are referable to *Shelbyoceras*, which in general physiognomy resembles *Plectronoceras*. The nautiloids appear to have reached a climax during the earliest Ordovician times and again near the close of the Lower Ordovician; the second climax is characterized particularly by the appearance of nautilicones. The majority of Cambro-Ordovician cephalopods are brevicones, but longicones are fairly abundant, and locally (in the Lower Ordovician) nautilicones are not particularly rare. Evolute specimens with closely spaced adoral septa and modified apertures are mature, rather than senile. Many Cambro-Ordovician cephalopods have orthochoanitic siphuncles, others holochoanitic. In the adapical portions of the siphuncle of certain Early Palaeozoic holochoanites (both brevicones and longicones) there are numerous closely spaced endocones, which are believed to have developed from diaphragms. The siphuncle of one genus of Lower Ordovician orthochoanites contains a central tube of unknown function. In another Lower Ordovician genus the walls of the siphuncular segments are solid-V-shaped in longitudinal section. The adapical portions of at least some of the early nautilicones show a siphonal caecum much like that of modern *Nautilus*, an orthochoanitic siphuncle, a small umbilical perforation, and a nepionic line near the end of the first volution. Both cameral and siphuncular deposits occur in Lower Ordovician nautilicones. Presumably brevicones gave rise to longicones and these in turn to gyroceracones and nautilicones. During the Lower Ordovician, holochoanites developed from orthochoanites; and cyrtochoanites, which in America at least are not known to exist before the Middle Ordovician, also evolved from orthochoanites.

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ERRATA

On p. 94 of this volume, 2nd col., lines 6 and 5 from below, *delete* 'has apparently... role of', *substituting* 'on experimental grounds denies their perception by'.

On p. 95, 1st col., line 5, *delete* 'therefore', *substituting* 'considered by some to be'.

Ditto, line 11, *after* 'records' *insert* 'angular accelerations and'.

Ditto, line 13, *for* 'sacculus' *write* 'utriculus'.

PROTEIN SYNTHESIS IN PLANTS

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Protein synthesis lies at the root of the most fundamental attributes of life: it is a major process in both growth and inheritance, since it underlies the building of protoplasm and the duplication of genes; it is the process involved in the multiplication of viruses and hence is the attribute by which the borderland of the living is distinguished from the non-living. In an indirect way too it must be related to the two outstanding characteristics of the structure of proteins. These are their diversity, which forms the central feature of the architecture of living matter and governs the endless variety of expression thereof; and their unity, upon which depend their common properties in different organisms of catalysing fundamental metabolic reactions.

These conceptions have largely determined the approach and scope of the present article. A comprehensive account of much of the past work on protein metabolism in the plant has been given by Chibnall (1939), and recapitulation of the ground covered by him has been avoided as far as is compatible with continuity of the theme. Chibnall, however, dwells largely from a biochemical point of view on the intermediate stages of protein metabolism, on the synthesis of amino-acids and the converse process of their deamination and disappearance in the respiratory sequence. In the present review consideration has been given rather more to the subsequent phase, that which we believe to consist of the linking of amino-acids to form protein; and an attempt has been made to view the process as a whole, from a somewhat different and more physiological angle, in its place in the living system. Evidence and ideas concerning the nature of protein synthesis come from biochemistry, from physiology, from cytology and even from mathematics. The greatest desideratum in this field of inquiry is perhaps that to a fuller extent the conclusions attained along one avenue of approach should become translatable in terms of those attained along another.

I. THE STRUCTURE OF THE PROTEIN MOLECULE

In the study of protein synthesis account should be taken of the structure of the protein molecule and of the complexes that it forms in the living cell. Already, in fact, as we shall see in the following pages, knowledge of such structure is giving direction to considerations of the synthetic mechanism. It is, therefore, desirable that we should begin with a review of the present position in this purely chemical field.

Although the complex structure of the protein molecule is still incompletely understood, the general principles involved are being brought to light by the development of certain new techniques; these are the use of the ultracentrifuge by Svedberg, refined methods of amino-acid analysis by Bergmann and his school, and X-ray investigations of protein molecules by Astbury and others. As a number of reviews of the advances resulting from these techniques have appeared (e.g. Taylor, 1937; Bernal, 1939; Astbury, 1939), only an orientating survey will be necessary here, with expansion of such terms as we shall require as foundations for subsequent discussion. Svedberg (1930) showed that the molecular weights of proteins fall into groups of comparatively small range. In each of these groups the values approximate to a multiple of 17,600: the molecules of higher weight seem to be association complexes, since they can be reversibly split with ease into units of submultiple weight. These facts suggest that there is some fundamental structural character common to all proteins; the range of values in each group implies, however, that the fundamental entity is not the molecular weight itself, but a quantity of which the molecular weight is a somewhat indefinite function. It is concluded that the proteins of each group have the same number of amino-acids, although the individual acids are different for each protein. Svedberg's work thus lays the foundation of the fundamental principle of the architecture of the protein molecule—constant structure with varying constitution.

The foundation for the belief in the constancy of the amino-acid number lies in the work of Bergmann & Niemann (1937, 1938), who conclude that there is a remarkable stoichiometric pattern in the protein molecule. According to this the total number of amino-acid residues in the molecule is $2^n \times 3^m$, where n and m are whole numbers; and the number of any individual amino-acid residues is $2^{n'} \times 3^{m'}$, where n' and m' may be either zero or a whole number. The total number of residues is a whole-number multiple of either 288, according to Bergmann & Niemann, or 72, according to Taylor (1937). These numerical rules lead to the assumption that the amino-acids are arranged in a definite sequence

in the protein molecule, the individuals recurring with frequencies determined by the exponents. Evidently there is a common ground plan for the architecture of the protein molecule and some factor that permits only certain discrete selections of amino-acids to be associated; this plan may find its interpretation in the requirements of stereochemistry, but it may well also be related to properties of the synthetic system.

The proteins occurring in plants belong generally to the group of so-called 'globular' proteins.* It is now generally believed that the molecules of these and other proteins consist not of simple polypeptide chains, but rather of chains that are in some way folded or held together by cross linkages. The results of X-ray studies (e.g. Bernal & Crowfoot, 1934) for example are incompatible with the long-chain polymer conception, and suggest rather a roughly spherical molecule. Unfolding of the molecule occurs on denaturation (cf. Mirsky & Pauling, 1936). This, in the light of X-ray data, probably does not involve very fundamental changes in the polypeptide structure and the molecular weight may in some cases be unaffected (Burk, 1937): unfolding occurs also in monolayers, and hence can result solely from the action of surface forces (Astbury, Bell, Gorter & v. Ormondt, 1938).

Various hypotheses have been put forward to explain the nature of this folding and linking of the protein molecule. The most detailed is the 'cyclol' hypothesis of Wrinch (1936, 1937, 1938, 1940). According to this, the protein molecule consists of a laminar, two-dimensional fabric, one residue thick, which may be folded to form a closed, polyhedral, cage-like structure; the essential feature of the hypothesis is that in this structure the polypeptide link is replaced by a lactam-lactim rearrangement. The cages can also adhere together reversibly either by hydroxyl bonds, by cysteine bridges or by salt linkages between residues carrying basic or acidic groups; in this way residue numbers that are multiples of 72 are predicted. It is a consequence of the cyclol structure that there are definite restrictions on the selections of residues and on the way in which they are arranged in the pattern: not all the α-carbon atoms have equivalent environments, so that a residue is attached only where the environment permits. This fact is held to explain the specific characters of the individual proteins, while the ground plan of the fabric is the common character of all proteins. The cyclol hypothesis has been developed on abstract mathematical foundations, but, nevertheless, in the minds of certain investigators furnishes an explanation of many of the properties of the protein molecule, particularly those which have been referred to in the foregoing paragraphs (Wrinch, 1937*b*, 1940). Wrinch & Jordan Lloyd (1936) have also suggested a correlative hypothesis in which closed polypeptides are linked into a fabric by 'hydrogen bonds'; the hypothesis could be developed similarly to the lactam-lactim postulate. The cyclol hypothesis, it should be added, has met with a mixed reception by laboratory workers; Bergmann & Niemann (1938) for example have condemned it as being incompatible with existing knowledge.

A quite different hypothesis of protein structure has been put forward by Mirsky & Pauling (1936) who use the hydrogen bond concept, but in a manner other than that of Wrinch & Jordan Lloyd. Mirsky & Pauling suggest that the molecule consists potentially of one or sometimes more polypeptide chains, which continue without interruption throughout the molecule; the chain is folded into a unique spatial configuration in which it is maintained by hydrogen bonds between the peptide nitrogen and oxygen atoms and also between the free amino- and carboxyl groups of the diamino and dicarboxyl amino-acid residues.

In addition to these complexes formed by linking of fundamental molecular units, other protein complexes are known to exist. These have been studied by Przyleçki (1940): they include compounds formed by salt linkages and also by primary and secondary valency bonds; the compounds are not only with other proteins, but with carbohydrates and with fats. It should also be pointed out that Sørensen (1930) and others have shown that certain proteins consist of units held together by secondary valencies, and that with appropriate change in the medium a rearrangement of the components takes place with precipitation of a relatively insoluble combination. In general it seems that protein molecules exist in the living cell largely as higher complexes formed either with themselves or with molecules of other compounds.

II. THE PATH OF SYNTHESIS

With this survey of the present outlook on the structure of the protein molecule or molecular complex, we may proceed to consider protein synthesis. There are two possible chemical paths along which this may take place: proteins are formed either from condensation of amino-acids or else by some sequence in which amino-acids do not participate. The evidence concerning which path is followed is mostly indirect, but it favours that involving amino-acid condensation. It is therefore proposed in this section to review the chemical sequence concerned in the amino-acid path and the evidence for believing that this sequence occurs in the plant; the alternative-path hypothesis will be referred to thereafter.

(1) *Amino-acid synthesis*

Amino-acids not only arise by protein breakdown, but are also synthesized in the plant from ammonia

* Banga & Szent-Gyorgyi (1940) have recently stated that fibrous proteins may also be of common occurrence in cells, and that they are found in chloroplasts.

and carbohydrate residues. This is suggestive evidence that they may be the direct building stones of proteins, but it is not proof, since, as Green (1937) has pointed out, only certain of many possible reactions are actually realized by the living cell. If, however, condensation of amino-acids occurs, their synthesis must represent the main point at which nitrogen and carbohydrate metabolisms are linked.

Our knowledge of the mechanism of amino-acid synthesis has been greatly extended by the work of Euler and his collaborators (Adler, Das, v. Euler & Heyman, 1938). They have found in higher plants a highly specific glutamic acid dehydrogenase; in the presence of this, ammonia, α-ketoglutaric acid and dehydro-cozymase, glutamic acid is formed reversibly. At the *p*H of living cells the end-point of the reaction is well on the side of synthesis; the reaction, however, is endothermic, energy being provided by a coupled oxidation. It has also been shown in animal tissues (Braunstein & Kritzmann, 1937, 1938) that the amino-group of glutamic acid can be transferred by an oxidation-reduction process known as 'transamination' to any α-ketonic acid with the formation of the corresponding amino-acid and α-ketoglutaric acid. Furthermore, the amino-group of any amino-acid except possibly glycine can be transferred to any α-ketonic acid provided either the amino-acid donator or the α-ketonic acid acceptor is a dicarboxylic acid. These transfers are reversible and on breakdown of amino-acids the amino-group goes over to α-ketoglutaric acid and glutamic acid is formed. Aspartic acid can also act as a starting point for transamination. If small amounts of an amino-dicarboxylic acid or an α-keto-dicarboxylic acid are present, the transfer of amino-nitrogen between two monocarboxylic acids is effected, the dicarboxylic acids acting as carriers in the transfer of amino-groups. In these transfers an enzyme 'transaminase' is concerned, the kinetics of which have been studied by Cohen (1940 *a*, *b*). Virtanen & Laine (1938) have demonstrated the transfer of the amino-group from aspartic acid to pyruvic acid with the formation of alanine in preparations of pea plants; it therefore appears that such processes occur in plants also. The two dicarboxylic α-amino-acids are regarded as entries and exits for ammonia in protein metabolism, oxalacetic and α-keto-glutaric acids arising in the normal course of sugar breakdown; and their frequent accumulation as amides on protein breakdown in the plant becomes more readily understandable. Aspartic acid can, it must be added, arise in the plant not only from oxalacetic acid and ammonia in the presence of aspartic acid dehydrase and cozymase (Adler *et al.* 1938), but also from fumaric acid by the action of aspartase (Virtanen & Tarnanen, 1932); indeed all amino-acids may be formed in the plant by *ad hoc* syntheses: but the fact that protein synthesis takes place readily at the expense of nitrogen stored as asparagine or glutamine suggests the importance of transamination. There is evidence (cf. Wood & Petrie, 1938; Wood, 1941) which suggests that there is a position of balance between amides and residual amino-acids of which the probable determinants are ammonia content, respiration rate and *p*H.

(2) *Amino-acid condensation*

Indirect evidence that proteins are naturally synthesized from amino-acids lies in the fact that proteolytic enzymes can effect amino-acid condensation in vitro. There is the synthesis of plastein from the products of peptic digestion after they have been highly concentrated and brought to *p*H 4·0 (see Wasteneys & Borsook, 1930). Plastein can be redigested by pepsin at *p*H 1·7. The number of amino-acids in its molecule is small, so that it is not to be regarded as a protein (Folley, 1933; Flosdorf, Mudd & Flosdorf, 1937; Flosdorf, 1941), but it is suggestive that the enzyme can catalyse the condensation process. Further evidence is provided by the work of Bergmann and his colleagues (Bergmann & Fraenkel-Conrat, 1937, 1938; Bergmann & Behrens, 1938; Bergmann & Fruton, 1938; etc.), in which simple peptides were synthesized by various proteinases from amino-acids, aniline, phenylhydrazine and other compounds in the presence of an activator such as cysteine. The optima of *p*H and temperature and the activator were the same as for hydrolysis by the same enzyme. The enzymes were found to have a remarkable specificity and to act on different peptide-like compounds differently. Thus, for example, papain hydrolyses benzoyl-leucyl-glycyl-glycine at the third peptide link, and benzoyl-leucyl-leucyl-glycine at the second link, but effects synthesis in a mixture of benzoyl leucine and leucine anilide. Small differences in the substrate composition determine whether and to what extent synthesis or hydrolysis takes place. Bergmann & Niemann (1937, 1938) consider that this type of specificity fits the proteinases for synthesizing the unique pattern of individual proteins. When a number of peptides and amino-acids are available, the enzymes subject them to a series of transformations by synthesis and hydrolysis, reconstructing one peptide bond after another, until a protein pattern is produced that is stable in the presence of the enzyme; this is the characteristic pattern of the particular protein molecule concerned.

(3) *The final stages in protein synthesis*

Whether the fabric structure of the protein molecule is synthesized directly, or whether simple polypeptides are formed first, is not known, but there is some ground for suggesting the second possibility. Many native proteins are known to be inaccessible to enzyme attack until they have been denatured; it is also believed that denaturation involves opening up of the ring or cage-like structure of the molecule into the chain condition, without necessarily any alteration in molecular weight,

Linderstrøm-Lang (1939) has therefore suggested that the secondary structure in the molecule blocks the peptide bonds to a certain degree, and that in protein breakdown a distinct, enzymatic or non-enzymatic initial reaction must take place prior to the fission of peptide bonds. The blocking may have its origin in steric hindrances or in the presence of chemical structures into which peptide bonds have themselves entered (e.g. cyclol fabrics). Linderstrøm-Lang, Hotchkiss & Johannsen (1938) suggest that in a solution of a globular protein there is an equilibrium between genuine and denatured proteins, and that trypsin, acting on the latter, would thereby result in progressive breakdown of the genuine protein. Denaturation, at least in its early stages, has also been shown to be reversible (Anson & Mirsky, 1931).

It may thereore be suggested that the final stage in the synthesis of the protein molecule consists of a reaction analogous to the reverse of denaturation, in which a complex structure is elaborated from the simple polypeptide chain. Added to this will be the formation of molecular complexes with proteins and other compounds (§ 1). Przyleçki (1940) quotes the case of fats, anchored to the protein by the basic groups of the side chains, spatially protecting the peptide linkage from attack. Probably then these complexes have also first to be broken down before protein hydrolysis can occur.

(4) *Alternative path hypotheses*

Despite this evidence supporting the hypothesis of synthesis from amino-acids, it is also necessary to consider the possibility that proteins are not built up by the reverse process to hydrolysis: there may be alternative paths of synthesis and hydrolysis and the tendency to proceed along one path in one direction may be opposed by the nature of the intracellular system. The apparent synthesis of proteins from amino-acids in tissues may thus involve preliminary deamination, the proteins then being built up along another path. It must be admitted, however, that the evidence for such alternative paths is slender.

Alcock (1936) somewhat sweepingly considers the amino-acid hypothesis untenable. He puts forward the hypothesis that protein synthesis commences with the formation of a simple unit, possibly by condensation of formaldehyde with ammonia or nitrous acid. This unit then polymerizes and internally differentiates, so that a definite proportion of each amino-acid is formed. The resulting molecule represents a primitive type of protein, the capacity to form which Alcock regards as a common attribute of living creatures. This simple protein then undergoes further differentiation, involving addition or removal of amino-acids or the addition of prosthetic groups, and resulting in the formation of the more complex proteins characteristic of living tissues. Block (1934) has put forward a somewhat similar hypothesis. He claims that there is a constancy of the relative ratio of the basic amino-acids in certain proteins and that these basic amino-acids constitute an *Anlage* which has a directive influence on the construction of the rest of the molecule. Bergmann & Niemann (1938) have criticized this hypothesis and question the accuracy of the supposed constant ratio. Jannsen (1939) has formulated a highly speculative hypothesis according to which proteins are synthesized by the linking up of half glucose molecules and the insertion in them of nitrogen atoms from previously existing nucleoproteins. Gregory & Sen (1937) believe that if synthesis were a reversal of hydrolysis the relationship between proteins and amino-acids would follow the mass-action law. The fact that it does not do so in a simple manner leads them to postulate a cycle of nitrogen metabolism in which the two processes are distinct and catalysed by separate enzyme systems. It will be made clear in § V (3) that the failure of the relationship to conform with such a mass-action conception can be explained in terms of the amino-acid path of synthesis.

The alternative path hypotheses are of a very speculative nature and are vague with regard to the stages traversed. For these reasons they are of less value as working hypotheses. The amino-acid hypothesis is more clearly outlined, possible synthetic mechanisms can be discussed in its terms, and suggestions can be made as to the mode of action of determining factors. For these reasons the present review is based largely on the amino-acid path as a working hypothesis. Should it at any time be found desirable to adopt an alternative hypothesis, the conceptions developed could be reorientated to conform therewith.

III. LOCI OF SYNTHESIS OF DIFFERENT PROTEINS

Concerning the types of protein in plants and their distribution in the cell we still know little. It is clear, however, that there are several different intracellular loci of synthesis. Thus synthesis must occur independently within the chloroplasts which may contain from 35 to 40 % of the total protein of leaves (Hanson, 1941; Hanson, Barrien & Wood, 1941); it seems improbable that proteins as such could pass into the chloroplast from the cytoplasm. It is also evident from the work of Hanson (1941) and Hanson *et al.* (1941) that the dynamics of gain and loss of protein differ to a certain degree in the chloroplast and in the cytoplasm (see § IV (1)). Chibnall (1939, p. 141) has shown that the protein of the chloroplast differs from that of the cytoplasm in having a lower content of lysine and a higher content of histidine; Hanson *et al.* have also shown in the case of Sudan grass that it has a considerably higher content of sulphur. Nezgovorov (1941) concludes from autolysis experiments that isolated chloroplasts contain proteases. It is obvious also that protein synthesis

occurs independently within the nucleus, where it is of a highly specialized character (see § IV (2)). Two types of nucleic acids occur in the plant: the first, desoxyribo- or thymonucleic acid, has as its sugar component *d*-2-desoxyribose; the second, ribo-, or yeast nucleic acid, has as its sugar component *d*-ribose (cf. Gulland, 1938). The former occurs in the nucleus, the latter, however, in the cytoplasm; this has been shown by Delaporte (1939) in yeast and by Behrens (1938) in the embryo of rye, and has also been shown to apply in amphibia (Brachet, 1938). Belozersky & Tschigirev (1936) conclude from their work on bean seedlings that, whereas thymonucleic acid occurs as true nucleoprotein in the nucleus, nucleoproteins isolated from the cytoplasm are artefacts, and that in the living state the yeast nucleic acid is free in the cytoplasm; this is in agreement with earlier work of Steudel & Takahata (1924) on yeast. According to Caspersson & Schultz (1940) the nucleolus contains ribonucleic acids.

Whether finally there is more than one kind of protein in the cytoplasm cannot yet be regarded as settled (Petrie & Williams, 1938). In seeds and other storage organs storage proteins are differentiated in an undissolved amorphous or crystalline state as aleurone grains; in leaves, however, according to Kostychev (1931) aleurone grains are absent. It might be suggested that all proteins in the cytoplasm of leaves must therefore play some part in metabolism; they must for example hold water by imbibition and adsorb ions. There are nevertheless certain observations that lend themselves to interpretation on the assumption that storage proteins exist in leaves. Petrie & Williams (1938) found that respiration rate of leaves, when expressed on the basis of total leaf protein, was higher when the leaves were more nitrogen-deficient; this would be explicable if storage proteins were piled up when larger amounts of nitrogen were available. Williams (1939) found a somewhat similar state of affairs for net assimilation rate. Wood (1941) considers the storage protein in these leaves to be chloroplast protein. There are, however, other ways in which these data could be interpreted than in terms of a differentiation of proteins.

IV. THE MECHANISM OF SYNTHESIS

(1) *Synthetic surfaces*

There is much evidence suggesting that synthesis, not only of proteins but of other metabolites, occurs at specialized surfaces in the cell. It is for example well known that prevention of proteolysis depends on the maintenance of the structural organization of the cell. Other evidence has recently been assembled by Oparin (1934, 1937) in whose school the theory has received considerable study. Oparin refers to various cases of apparent variation in the hydrolytic activity of enzymes which could be correlated with possible changes in the colloidal state of the protoplasm; he includes such effects as those of temperature on invertase action and of inorganic ions on amylase activity in living cells. He demonstrates the fact that enzymes are readily adsorbed on proteins, in which case they become inactive, and quotes evidence that in seeds and leaves there is an adsorbed inactive fraction of amylase, the active fraction being readily soluble (Oparin & Riskina, 1932; cf. also discussion by Kertesz, 1937). Upon this foundation he puts forward the hypothesis that the adsorbed enzyme is responsible for synthesis and the free fraction for hydrolysis (see also Kursanov, 1936). For the particular case of proteolytic enzymes this suggestion is invoked by Kursanov & Bruishkova (1938) to explain results obtained by infiltrating leaves with protein hydrolysates. Most of the experimental work connected with the hypothesis employs infiltration, a technique to which objections might be raised. The chief work designed directly to test the hypothesis is that of Kursanov (1936) who infiltrated leaves with various doses of invertase, together with either sucrose or glucose-fructose mixtures. Measures of the rates of both the synthetic and hydrolytic reactions were thus obtained. It was found that small doses of invertase accelerated the rates of both synthesis and hydrolysis; but larger doses resulted in only the hydrolytic process being increased. From this Kursanov concluded that with increasing doses the surfaces at which sucrose is synthesized became fully saturated with enzyme, after which no further increase in rate of synthesis was possible; on the contrary, invertase would continue to accumulate in the continuous phase where hydrolysis would thus be further increased in rate. Kursanov then induced proteolysis in the leaves either by exclusion of oxygen or by supplying papayotin and cystein. This resulted in a decreased rate of synthesis of sucrose and an increased rate of hydrolysis. Kursanov concluded that the proteolytic activity had resulted in breakdown of the protoplasmic constituents on which the invertase was adsorbed. Finally he found that on complete autolysis of the tissue, the hydrolytic activity increased eight times. Inactivation of the enzyme by other phenomena than adsorption needs also to be considered.

The hypothesis is plausible, and deserves study if practicable by more refined experimental methods. Oparin supports it by incorporation of the earlier developed view that mitochondria are related to enzymatic activity. It has long been deduced from cytological observations that various synthetic processes occur within or at the surface of mitochondria. It has also been shown by Horning & Petrie (1927) and confirmed by Newcomer (1940) that starch hydrolysis in germinating cereal grains is associated with dissolution of mitochondria in close association with the starch granules. Horning & Petrie suggested that under these circumstances amylase was released from the mitochondria. In this connexion various facts can be cited which are

suggestive although they can scarcely yet be integrated into a definite concept. Chrzaszcz & Janicki (1933) give grounds for suggesting that the release of inactive amylase in germinating grains is the result of the activity of proteolytic enzymes, which might conceivably hydrolyse a protein adsorbent. That mitochondria are partly composed of protein has been confirmed analytically by Bensley (1937). Linderstrøm-Lang & Holter (1934) centrifuged eggs of *Psammechinus* and found the greater part of the dipeptidase activity to be resident in the heavier layer containing the mitochondria, although other granular components of the cytoplasm were also present. In other marine ova and in *Amoeba*, however, the peptidase was found to be diffusely distributed in the hyaline plasm or matrix of the cytoplasm (Holter, 1936; Holter & Kopac, 1937). Joyet-Lavergne (for references see Petrie & Williams, 1938, p. 354), on the basis of a reaction with oxidation-reduction indicators, believes that mitochondria are also the seat of respiration, which would be of interest since the sequence of protein synthesis is coupled with respiration at least at one point (cf. §§ IV (3) and V (4)). It should be added, however, that Joyet-Lavergne's interpretation of his results has been subjected to criticism on various grounds.

(2) *The concept of the protein template*

Granted that synthesis occurs at intracellular surfaces, themselves possibly composed at least in part of protein, we have now to consider more closely the mechanism and directive forces of the process occurring there, as distinct from the mere categorizing of the biochemical sequence which was our task in § III. Here we shall be concerned more particularly with the post-amino-acid stage, since little is known of the sites of amino-acid synthesis.

Wrinch (1937*a*, 1938) has given some consideration to this subject in the light of the cyclol hypothesis. The cyclol pattern is a dorsiventral one, with the side chains emerging from one surface only. She therefore suggests that protein synthesis is a surface phenomenon in which new material is built into a pattern determined by that of the surface, much as the outer layer of atoms in a growing crystal is built into a pattern determined by the surface lattice of the underlying layer. This involves in the case of the globular proteins, as Langmuir & Schaefer (1938) point out, an opening out of the cage-like structure into a two-dimensional monolayer. The conditions that might lead to the formation of a template of this kind are of course unknown. Support for the concept is found, however, in the autocatalytic formation of pepsin and trypsin (Northrop, 1937) and also in the autosynthesis of genes and viruses. The concept is actually independent of any particular hypothesis of the structure of the protein molecule, and had been put forward, prior to Wrinch's formulation, for the specific cases of gene and virus multiplication. Concerning these cases speculation has been more detailed than for the case of the cytoplasmic proteins, as will be seen from the following review.

The suggestion is (Muller, 1937; Haldane, 1937; Snell, 1938; Gulick, 1938) that gene synthesis occurs from amino-acids and other components present in the medium surrounding the chromosomes. Each gene attracts these components at specific loci on its surface, which contains molecular configurations similar to, or identical with, that of the adsorbed molecules. A repetition of the identical pattern thus results, with the formation of a daughter chromosome. The attraction of the identical chemical units is likened, as in Wrinch's suggestion, to the attraction in crystal growth, where the units are, however, held by secondary valency bonds. When so aggregated, the new units are in a favourable position to be linked together to form the daughter gene. Snell also compares chromosome reproduction to the mechanism of the growth of cellulose walls suggested by Sponsler (1929). According to this the cellulose molecules of the wall attract glucose molecules from the cytoplasm, which then condense and form a new layer of cellulose. Sponsler stresses the presence in the cellulose surfaces of regularly arranged β-glucose residues, which suggests a comparison with crystal surfaces in which large molecules act as structural units, and concludes that the forces of crystallization form a part of the mechanism by which glucose molecules become converted into cellulose.

An analogous method of autosynthesis has been suggested in the case of viruses by Stanley (1938). According to this the virus particle is able, through its structure and surface forces, to attract and orientate at its surface component parts from the cytoplasm of the host cell. It is suggested that, since the intact virus particle represents a more stable form, there will be a mutual solvation of forces with the formation of a new intact molecule. A similar mechanism has been outlined by Best (1939).

Clearly in each of these cases the template must be two-dimensional; a three-dimensional fabric cannot be visualized as imposing its pattern on a new molecule. Wrinch (1940*b*) has discussed this point; she attributes to the gene a cage structure which in itself may be unstable on account of the high proportion of basic side chains, but which may be stabilized by the presence of nucleic acid. Instability of a cyclol cage may lead to a tearing of the structure resulting in the formation of a two-dimensional fabric. Wrinch therefore suggests that in gene synthesis a change of *p*H or other disturbance destroys the balance of protein and nucleic acid, leading to opening of the closed fabric to form a two-dimensional template.

Caspersson (1939 *a*, *b*) and Caspersson & Schultz (1938) have demonstrated certain relations between nucleic acid production and nuclear division which lead them to suggest that the capacity of cell repro-

duction in genes and viruses may depend on the nucleic acid portion of the nucleus, and that the property of a protein that allows it to reproduce itself is its ability to synthesize nucleic acid. Caspersson & Schultz (1938) have also shown that the cytoplasm of rapidly dividing animal and plant cells has a high content of substances having properties of pentose nucleic acids, and that the content of these substances decreases in later developmental states.

It is conceivable that plastid-protein formation may also be autosynthetic, since these organs have an autonomy similar to that of the nucleus. That the same applies to the cytoplasmic proteins seems less likely. Gulick (1939) has suggested that many proteins must be very weak in their capacity for autosynthesis, and that their production is consequently dependent on a distinctive enzyme instead of on a pattern molecule. There is no allowance for enzyme participation in the template hypothesis, and in the case of virus synthesis, at least, there is no evidence that enzyme activity is concerned. Associated with this is the apparent irreversibility of virus synthesis: even under conditions of nitrogen starvation it appears that the plant does not draw on virus protein (Spencer, 1941). Finally there is no evidence yet that cytoplasmic protein synthesis is always concerned with nucleoproteins. It thus seems that if the template hypotheses put forward for the gene and virus are accepted, it becomes necessary to allow for the synthesis of cytoplasmic proteins being of a fundamentally different character.

(3) *The thermodynamics of protein synthesis*

The synthesis of amino-acids, as was pointed out in § II (1), is coupled with the respiratory process, whereby it obtains its necessary energy. This in fact is the main point at which energy enters into the synthetic sequence. With regard to the subsequent stages the state of affairs is less clear. In a mixture of amino-acids and proteins and the appropriate enzyme the position of equilibrium is usually at almost complete hydrolysis; amino-acid condensation must therefore also involve an increase in the free energy of the system. At present, however, we know almost nothing of the manner in which energy is provided, although certain speculations can be put forward. Synthesis could occur if the product were insoluble or removed, spatially. Thus Bergmann's in vitro syntheses (§ II (2)) are explained by the insolubility of the products* (Fruton, 1938; Bergmann, 1939); here clearly the energy necessary for the synthesis would be obtained from the heat content of the environment or from the heat of precipitation of the proteins. It would be unjustifiable, however, to regard the cytoplasmic proteins as being generally out of solution, although this principle might account for the continued formation of insoluble storage proteins in seeds.

It has already been pointed out (§ II (3)) that the final stage of protein synthesis may be the establishment of a complex structure in the molecule, comprising the formation of cross-linkages and the linking together of molecules to form aggregates either with further protein molecules or with those of other compounds. It has also been pointed out that these complex molecules or aggregates are frequently inaccessible to attack by proteolytic enzymes. It is therefore possible that the energy necessary for the maintenance of the apparently supra-equilibrium amounts of protein in the cell is that which is used in the establishment of these secondary linkages. It has been pointed out in § II (3) that this final stage may be analogous to the reverse process of denaturation; it is also evident from thermodynamical considerations (Mirsky & Pauling, 1936) that energy would be required to effect such a reverse process. Frank (1936) states that the formation of the cyclol monolayer in itself is a stabilizing process, although the formation of the monolayer alone does not provide sufficient energy to result in the equilibrium being far on the right-hand side of the equation. An additional source of energy, however, he points out, is provided by hydration, which is well known to stabilize hydroxylic tautomeric forms. Conditions tending to dehydration would in such case render proteins liable to revert to the open-chain structure, in which state they would be liable to transformations such as denaturation and to attack by proteolytic enzymes.

A further possibility is that for protein synthesis, as for amino-acid synthesis, energy is obtained from simultaneously occurring oxidative reactions. How the coupling of the endothermic reaction with the oxidative process might be achieved is, like the whole problem of biochemical energy transfer, highly obscure. It is conceivable that the energy transfer is not even a directly chemical one: the energy may be used in maintaining a structural pattern in which proteins are protected from enzyme attack, as in the possibilities discussed above; or the energy might be concerned in the control of the activities of the enzymes themselves, a possibility in harmony with Oparin's views (§ IV (1)). In the words of A. V. Hill (1929), '...it seems inevitable, from the fact that oxygen can prohibit reactions which take place in its absence, that it is being used in maintaining the integrity of boundaries or interfaces, perhaps material ones, perhaps dynamic equilibria of a non-geometrical kind, by which things are held apart'. Many years ago Warburg (1914)

* Bergmann (1939) has discussed the thermodynamics of protein synthesis. He cites the example of the enzymatic linking of acetyl phenylalanylglycine and glycyl leucine being continued until the exhaustion of the substrate; the driving force is here the further continuous breakdown of the product, under the influence of the same enzyme (papain) but along another path, to the first-mentioned reactant, leucine and glycine. The example, however, is not an explanation of the biological accumulation of proteins since the net change in the system is one of degradation.

was struck by the fact that living cells consume energy even when performing no external work, and suggested that in so doing they are maintaining intracellular structures. The relation of respiration and oxygen supply to protein synthesis is subject to experimental attack, and will be considered in § V (4).

V. THE REGULATION OF SYNTHESIS

(1) *The concept of the steady state*

From the considerations of the previous section it seems likely that the proteins present in the normal cell are maintained there by a constant dissipation of energy. The system clearly cannot be one of equilibrium. Such a system, nevertheless, can theoretically attain a 'steady state', which is one where the concentrations of reactants and products and the rate of energy consumption are all constant (cf. Lotka, 1925; Hill, 1929, 1930, 1931). Whether such a state is in reality ever attained in the nitrogen metabolism of a growing plant, even in a constant environment, is doubtful: but it is possible that under certain conditions there may be established a 'drifting steady state'; by this is implied that change in the determinants of the state is so slow that a succession of states results, each of which is inappreciably removed from a steady one. Study of the interrelations between reactants in metabolism must be based on the appreciation of this fundamental principle. We must seek for the factors that determine these steady states to which we believe the cell, in maintaining its high internal concentrations of protein, approaches even although conditions prevent its attaining them. We may thus expect the amounts of a metabolic product to be related to the concentrations of the reactants and also to certain parameters relating directly or indirectly to the energy release. These parameters are the regulators of the metabolic processes concerned.

These principles are of particular value in approaching the problem of the regulation of protein synthesis. We must first, however, consider briefly our limited knowledge of the regulation of amino-acid synthesis; here it is less certain to what extent we are concerned with non-equilibrium states, although they are doubtless indirectly concerned. In so treating the subject we are again confining our consideration to the hypothesis of the amino-acid path. It is more difficult to suggest how regulation might be achieved along the nebulous alternative paths; if subsequently it is necessary or desired, many concepts concerning regulation developed in this section could be easily re-orientated to harmonize with an alternative-path hypothesis.

(2) *Regulation of amino-acid synthesis*

As to the regulation of this early stage in protein synthesis we can do little more than present a hypothetical picture developed from a broad consideration of biochemical and physiological investigations. Its detailed substantiation calls for controlled physiological experiments in which the behaviour of the products of intermediary metabolism is observed. In connexion with the hypothesis, reference should be made to the lengthy discussion of amino-acid synthesis and breakdown in the book of Chibnall (1939) and in particular to the discussion therein of the work of Yemm and Vickery *et al.*

At the steady state respiration proceeds through the four-carbon acid path at a constant rate and the amino-acid level is unchanged. An increase in the concentration of α-ketoglutaric or oxalacetic acid or ammonia will increase the amino-acid concentration. Wood & Petrie (1938) have shown that the curve relating amino-acid content of leaves to the ammonia content is concave to the ammonia axis, a type of relation to be expected if the other reactant is limiting. Wood & Petrie and others have also found that respiration rate and amino-acid content are correlated. Since respiration is involved both in the amination and in the deamination processes of glutamic and aspartic acid (§ II (1)) it is perhaps to be expected that high concentrations of amino-acids would tend to maintain a high respiration rate. It does not seem to be an argument in favour of an alternative path hypothesis of protein synthesis as Gregory & Sen (1937) suppose.

If the carbohydrate level, or rather the rate of 'triosis', falls, then it must be expected that the four-carbon acids will decrease in concentration, the energy supply will fall, and deamination will occur, bringing proteins ultimately into the respiratory cycle. This appears to have occurred in the isolated barley leaves used by Yemm (1935, 1937). Yemm's work suffers from the fact that the water content of the leaves probably fell under the conditions of his experiments, and this, as will be seen in § V (6), would be expected in itself to cause protein breakdown. It is obvious from Yemm's data that protein breakdown and amino-acid accumulation occurred in excess of respiration requirements. As a natural consequence of adjustment of positions of balance, the amino-acids were partly converted into amides, the balance moving further towards amides as carbohydrates became exhausted and the carbon residues of the amino-acids were respired.

It is obvious nevertheless from work of Vickery, Pucher, Wakeman & Leavenworth (1937) that, even when the water content of isolated leaves is maintained constant, a similar breakdown occurs although it is of less magnitude. This is independent of carbohydrate exhaustion since it also occurs when the leaves are illuminated and carbohydrates accumulate. The point will be further discussed in § V (7). The constancy of the malic and citric acid contents in the illuminated leaves confirms the opening statement of the foregoing discussion.

(3) *Relation of protein synthesis to amino-acid concentration*

We must begin consideration of the subsequent phase of protein synthesis with an inquiry whether a relationship exists between the amounts of proteins and amino-acids in plant tissues. Such a relation has in fact been found by Petrie & Wood (1938*a*, *b*), using total leaves of gramineous plants, to be well marked, and characterized by concavity* to the amino-acid axis; and it seems probable that this is the type of relation existing at the steady state. Petrie & Wood obtained the data from which this relation was derived over as brief a period as possible, generally amounting to three days. This was done to minimize ontogenetic changes in the system, particularly in its extensive properties. A steady state relationship cannot be perceived if the system increases during the period of collection of the data; nor are data on a dry-weight basis comparable if the dry weight is undergoing change. Much experimental work has been described which would have been of considerably greater value had an awareness of these facts influenced its design (cf. also Walkley & Petrie, 1941). The form of the relation suggests that, as amino-acid concentration increases, some other factor becomes limiting. The nature of this factor is unknown; but the evidence suggests that the form of the relation is determined by a number of factors through which the protein metabolism of the plant is regulated.† Consideration of these will form the remaining task of this review.

One such factor is directly associated with the amino-acid concentration. It has been pointed out by Petrie & Wood (1938*b*) that a given relation of proteins to amino-acids will hold only so long as the proportions between the individual amino-acids remain constant; the relation will be different for every change in amino-acid proportions. Petrie & Wood suggested that the concavity of the curve relating the amounts of proteins and amino-acids might indeed be due to the fact that certain amino-acids increased less rapidly than others when the total concentration was increased; and they showed that cystine was one amino-acid that, under their experimental conditions, did behave in this way. Lugg & Weller (1941) have shown what may be an application of this principle where protein synthesis appears to be limited by the amount of methionine present in the seed. It is possible, however, that transamination (§ II (1)) occurs at the centres of protein synthesis, so that the protein level maintained in the cell may be largely independent of the proportions among the free amino-acids in the cell.

(4) *Relation of protein synthesis to respiration rate*

If energy is constantly expended in maintaining the protein content of the cell it is worth while to seek for a correlation between the protein content and the respiration rate of the tissue. The absence of such correlation would not, however, disprove the participation of an oxidative process, since the total respiration may be made up of a number of components, not all of which are related to protein synthesis and not all of which vary in the same way. We are unable at present to separate such components or to say how they could be associated with amino-acid condensation.

Several recent publications bear upon the relation of protein synthesis to respiration. Steward & Preston (1940, 1941) and Steward, Stout & Preston (1940) have studied disks of potato tuber immersed in aerated solutions of chlorides, bromides and nitrates of potassium and calcium, and sulphate of potassium. They found that respiration rate was increased by increasing the external concentration of potassium salts and decreased by increasing the external concentration of calcium salts. Effects similar in direction were produced on protein synthesis, except that calcium nitrate increased protein synthesis although it depressed respiration. The writers draw from their data the following conclusions: (*a*) In the chlorides, bromides and sulphates, synthesis occurs from amino-acids, not directly, but after oxidative deamination; hence is explained the relation between the amount of carbon dioxide respired and the amount of protein synthesized. The variations in amount of synthesis are regarded as direct effects of the salt ions on the synthetic process. (*b*) Since with calcium nitrate, and to a less extent with potassium nitrate, the respiration is not increased in conformity with the large amount of protein synthesis occurring, synthesis was from the inorganic source and not from the amino-acids, and hence deamination was not involved. (*c*) In the light of variations in its content, glutamine or some similar labile amide is an intermediate in the formation of proteins. Conclusions (*a*) and (*b*) are compatible with the ideas so far developed in this review. The amino-acids stored may not be those required for synthesis and exchange amination or deamination may occur prior to synthesis; whether however exchange amination would involve increased respiration rate does not yet seem to be clear. It is possible also that the amino-acids synthesized directly from nitrate are those more specifically required for protein synthesis. Conclusion (*c*), on the other hand,

* Were the system one in which equilibrium existed it would be expected that the curve would be convex (cf. Wasteneys & Borsook, 1930) provided that all amino-acids varied together in concentration.

† It may be noted in passing that Gregory & Sen (1937) were unable to believe such a system as this possible. According to them the mass action law necessitates that 'relative concentrations of proteins and amino-acids would tend to constant values, and an increase in amino N relative to protein N could only result from an increase in concentration of the stage previous to amino-acid formation'. This, as mentioned in § II (4), leads them to reject the hypothesis that synthesis is a reversal of proteolysis.

is doubtful: variations in glutamine content may indicate nothing more than a change in the position of balance between amides and other amino-acids, and indicate nothing as to the path of synthesis.

For the purpose of drawing conclusions concerning the mechanism of protein synthesis the data of Steward *et al.* are not altogether satisfactory, because it is evident that cell division was active at the surfaces of the disks. The relation between cell division and protein synthesis is obscure, but it can be conceived that the direct effect of the treatments might have been towards hormone production in varying amounts, causing different capacities for or rates of cell division; this hormone may simultaneously influence protein synthesis. Thus it is uncertain whether it can be concluded that the treatments have direct effects on protein synthesis such as might occur in mature, somatic cells. Cell division certainly brings high respiration rate in its train, but it does not follow that this is due to deamination. The amount of protein synthesis may have been determined by the capacity of the system to increase by cell division. Steward & Preston stress the relation between the amount of carbon dioxide respired in a certain time and the amount of protein synthesized. They state: 'The conditions which obtain in the aerated, cut discs... so encourage synthesis that any hypothetical tendency to simultaneous breakdown is masked and evidence for a continuous cycle of nitrogen metabolism is lacking.' It seems, however, inconsistent with our knowledge of the nature of metabolic processes to view protein synthesis otherwise than in terms of balanced reactions.* Steward & Preston's data do not preclude the possibility that synthesis and hydrolysis were simultaneously proceeding. It therefore seems reasonable to interpret the data by concluding that treatment indirectly changes the position of balance between proteins and amino-acids. If the maintenance of the position of balance involves expenditure of energy, a plausible interpretation is that the treatment affects the position of balance through its influence on the respiration rate.

Apart from the continuous change in the system owing to cell division, there are several reasons why the data are unsuitable for testing this interpretation. Respiration rate is summed over a long period and the values recorded are therefore not necessarily measures of the instantaneous rates at the time of estimation of the protein and amino-acid contents; the distribution of the latter compounds throughout the tissue must be very uneven and also must drift, and the average contents of the whole tissue may not be indices of the position of balance in the surface cells where presumably most of the freshly formed protein is present. Nevertheless it is interesting to find that, for the data pertaining to the chlorides and nitrates, the protein content (P) is largely determined by the amino-acid content (A) and the respiration rate (R). A regression of the form $P=a+b_1A+b_2R+b_3R^2$ accounts for 81 % of the variance of the protein-nitrogen content. The data for the bromides were omitted from the calculation, since it is clear that the bromide ion raises the protein content to a much higher level than can be accounted for by the regression derived from the other data; the sulphate data were also omitted because they are incomplete. No effects of the remaining ions other than those on amino-acid content and respiration rate are evident. It is thus likely that only one type of synthesis is concerned, namely the condensation of amino-acid into protein, and the rate of this is connected with the rate of carbon dioxide production. The results with calcium nitrate fit adequately into this picture without the not altogether attractive assumption that calcium 'diverts protein synthesis from the amino-acids and encourages it to proceed without that oxidative deamination of amino-acids.' It would be unwise, however, to attach too much significance to a relation between protein synthesis and respiration rate in a system in which cell division is occurring.

It should finally be pointed out that in the work of Steward *et al.* (1940) a unique relation was found between the amount of protein synthesized and the respiration rate. From this relation an extrapolation was performed to find the amount of carbon dioxide respired when no protein was synthesized. The extrapolation is a dangerous one, since the form attributed to the relation depends largely on the accuracy of an isolated observation at a low respiration rate. Nevertheless, on this basis the writers divide respiration into two components, one of which is supposedly linked with protein synthesis and the other of which is independent of it; and they attach theoretical significance to their relative proportions. They have not considered the possible effect of change in amino-acid content on the proportions.

Quite a different approach to the present problem lies in the work of Gregory & Sen (1937), in which correlations were found between respiration rate and various chemical entities in mature leaves of barley grown with various nutrient supplies; all attributes were expressed on a dry-weight basis. It was found that respiration rate was highly correlated with both protein and amino-acid content. Both the latter quantities were highly correlated in themselves, and it was therefore difficult to disentangle their separate relations to the respiration rate; the data suggested, however, that respiration rate was most closely related to amino-acid content, except that, when this was high, the rate was limited by carbohydrate deficiency. Unfortunately the value of Gregory and Sen's work is reduced by the combination of data relating to different positions of insertion of the leaves on the primary shoot and to different past

* Work by Vickery, Pucher, Schoenheimer & Rittenberg (1940) with labelled nitrogen provides strong evidence that there is a continuous building up and breaking down of proteins in plant tissues.

histories resulting from differences in nutrient supply. It is evident that the leaves must have varied greatly in structure. Variations in the amount of cell-wall material in leaves will clearly tend to cause positive correlations between protoplasmic attributes such as contents of chemical entities and respiration rate (Petrie & Williams, 1938). Stated somewhat differently, correlations may occur between respiration rate and protein content because the latter is a rough measure of the respiratory seats;* this, it might be added, could also have contributed to the correlation shown in the work of Steward & Preston (1941). These facts were, however, taken into account in a subsequent investigation from Gregory's school by Richards (1938). The latter, like Petrie & Williams, placed his respiration data on a protein basis, which eliminated much of the position and treatment effects; the remaining variability is largely accounted for in terms of reducing sugar content. Richards was struck by the extraordinary constancy of respiration rate on a protein basis. Being unable to believe that factors such as phosphate deficiency and amino-acid content have no effect on respiration, he rejects the simple concept of protein being only a measure of the mass of respiring protoplasm, or of the enzyme systems involved, and concludes that there must be a reciprocal relation between the two variables, each being dependent in some measure on the other. Protein content may then, he concludes, be partly dependent on respiration rate. The respiration data of Gregory & Sen expressed on a protein basis show a rise with nitrogen or potassium deficiency, although Richards's data show that there is no rise with phosphorus deficiency. Richards therefore suggests that a minimal respiration rate may be necessary to maintain a given protein level. The rate may rise above this under certain conditions of manurial deficiency, when presumably other factors limit protein synthesis. When phosphorus is limiting, the respiration rate is lowered below the critical level and the protein level consequently also falls.

It is evident that the question whether the protein-amino-acid balance is affected by respiration rate is not yet answered. Further investigation is needed, especially of a type in which respiration rate is altered by varying the oxygen supply.

(5) *Relation of protein synthesis to oxygen supply*

Mothes (1933 *a*, *b*) has carried out extensive investigations on the regulation of protein metabolism in leaves. He showed inter alia that when protein hydrolysates were injected into leaves in the dark net protein synthesis occurred in pure oxygen, but no appreciable change in protein content occurred in air, while if the oxygen content of the atmosphere was low there was net hydrolysis. Mothes suggested that the activity of the proteolytic enzymes in the leaf is regulated by sulphydryl compounds. In oxygen deficiency these are reduced; and it is well known that in this state these compounds activate proteolytic enzymes in vitro. Here it might be pointed out that the concept of activation in the living system requires clarification. In an in vitro system proceeding to equilibrium at complete hydrolysis, activation will increase the rate of hydrolysis. Such an activation would not necessarily affect a position of balance in the living system unless synthesis were a process distinct from hydrolysis either chemically as in the alternative path hypotheses (§ II (4)) or spatially as in the hypothesis of Oparin (§ IV (1)).

Paech (1935) has adversely criticized Mothes's work. Paech found that in the absence of oxygen protein hydrolysis in leaves did not usually commence until injury had set in; it occurred prior to this only if injury was very late in appearing, and in this case he considered carbohydrate exhaustion to be the cause of the protein breakdown. Paech pointed out that proteolytic enzymes are normally protected from oxygen inactivation in the cell, but that as soon as a tissue becomes injured the enzymes become susceptible to the influence of oxygen and autolysis will then depend on the degree to which oxygen is excluded. Mothes (1936, p. 227) has replied to Paech, but it is evident that a further critical investigation of the relation of oxygen tension and respiration rate to the position of balance between proteins and amino-acids is greatly needed.

(6) *Water content as a factor in nitrogen metabolism*

Decrease in water content of a tissue appears to increase the rate of certain hydrolytic processes in plant metabolism. Numerous workers have shown this in the case of starch hydrolysis (cf. Amos & Wood, 1939); Oparin (1937) claims that decreasing water content of leaves causes increase in the hydrolytic activity of invertase; and the same effect was shown in the case of protein hydrolysis by Mothes (1931). The latter effect has been further investigated by Petrie & Wood (1938) in the work referred to in § V (3). It was found that, when the water content and nitrogen supply of the total leaves of gramineous plants were varied, the values of the protein content could be almost completely predicted in terms of the contents of amino-acids and water. Reasons were given for believing that the relationship held at the steady state. In marked contrast to this relationship between the contents of protein and water, Wood & Petrie (1938) found that on the whole water content did not appear to affect the amino-acid or amide contents; in other words the

* It is of interest that when the respiration data of Gregory & Sen are placed on a protein basis they show a relation to nitrogen treatment similar to that found by Petrie & Williams.

relation of the contents of these compounds to those of their precursor, ammonia, was generally independent of the water content of the tissue. Petrie & Wood have pointed out that while water is a factor in the relation between proteins and amino-acids on a dry-weight basis, we do not know whether this applies also on a concentration basis. The relation at constant water content, which may be assumed to be equivalent to the relation on a concentration basis, is concave to the amino-acid axis. Petrie & Wood hence showed that an increase in concentration of the two variables due to decrease in water content would produce the observed effect on their amounts expressed on a dry-weight basis. Therefore, although it is certainly probable, it is not yet certain that water content affects the velocity constants of synthesis or hydrolysis. Oparin (1937) considers, in terms of his theory outlined in § IV (1), that decrease in water content causes release of adsorbed enzyme into the continuous phase where it effects hydrolysis. It is also possible that dehydration causes an opening up of the cage-like structure of the protein molecule (§ I) resulting in its becoming more accessible to enzymatic breakdown.

(7) *Relation of protein synthesis to carbohydrate content*

Paech (1935), after considering his own and other data, came to the conclusion that the protein level in a plant organ is determined by the content of total nitrogen and monosaccharides according to the mass-action principle. Paech's work has been carefully discussed by Chibnall and therefore need not be reconsidered here. To some extent such a relationship must hold, but nevertheless an exact relationship can hold only between the immediate reactants and products. Amino-acid content may be determined by the α-ketonic-acid content, which will not always vary with the content of monosaccharides. There is no evidence yet that carbohydrates have any direct or indirect relationship to the balance between proteins and amino-acids.

(8) *Relation of nitrogen metabolism to mineral elements*

We have little knowledge of the effect of mineral elements on the early stages of protein synthesis, although it appears that phosphorus deficiency increases the ratio of amide to amino-acid nitrogen (Richards & Templeman, 1936). There is evidence, however, suggesting that mineral elements may have a marked effect on amino-acid condensation. This is claimed to be the case for potassium by Richards & Templeman. Their work suffers, like that of Gregory & Sen (§ V (4)), from the fact that relationships among amounts of metabolites are compared on a dry-weight basis in structurally non-uniform material. Richards & Templeman found that potassium accumulates first in the meristems and is then withdrawn from them in the case of deficiency. When the withdrawal occurs, proteins break down and the amino-acid and amide contents increase. Richards & Templeman regard this as evidence for concluding that the effect of potassium is on the breakdown of proteins rather than on the reverse process. They go on to say that if inability to synthesize proteins is the primary cause of the reduction in growth and of the high amino-nitrogen concentration in potassium-deficient plants, amino-acid accumulation would be one of the first symptoms of deficiency, and not, as the evidence suggests, one of the last. If, however, we regard synthesis and hydrolysis as two simultaneously occurring, opposed processes, effects on them separately cannot be distinguished in this way. The time of onset of amino-acid accumulation can indicate little more than the time when the effect of the deficiency becomes acute. All that can be concluded from Richards & Templeman's data is that potassium supply may affect the position of balance between proteins and amino-acids; the evidence for this would be more convincing could it be obtained from structurally uniform material, as, for example, by a technique based on that of Walkley & Petrie (1941). Incidentally the work of Petrie & Wood (1938 *a*, *b*) and Walkley (1940) suggests that the rate of net protein breakdown may have little effect on the amount of amino-acids in leaves; the latter quantity probably depends largely on the magnitude of sinks external to the leaf concerned. Richards & Templeman further suggest that the influence of potassium on protein synthesis is secondary and consists in the maintenance of some essential process or constituents of the protoplasmic complex. This is a plausible hypothesis, but it is a big assumption to say, as they do, that 'Hydrolysis is due to premature ageing and death rather than vice versa'. We are scarcely in a position to decide yet whether senescence can be caused otherwise than through withdrawal of amino-acids from the leaf or through a change in the steady-state relation between proteins and amino-acids (cf. Walkley, 1940; Walkley & Petrie, 1941).

Williams (1938) and other workers quoted by him have shown that nitrogen accumulates in the form of amino-acids and other soluble forms to a greater extent in phosphorus-deficient plants. This suggests that increasing phosphorus supply increases the net rate of synthesis from a given concentration of amino-acids. This conclusion, however, like that drawn in the case of potassium, requires to be substantiated by experiments on initially uniform material.

VI. SUMMARY

The final stages in the formation of protein, rather than the formation of amino-acids, are discussed. The approach is physiological rather than biochemical. After reviewing

the present state of knowledge of the structure of proteins various suggestions as to the substances used in the formation of the protein are considered. The evidence seems to be in favour of the formation by condensation of amino-acids rather than by the polymerization of some simple unit. This leads to a consideration of the various seats of synthesis in the cell and to the mechanism by which the proteins are formed from their constituent parts. The article concludes with a discussion of the interrelation of nitrogen metabolism and respiration and of the factors governing amino-acid formation and protein synthesis.

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THE NUTRITION OF THE PROTOZOA

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I. INTRODUCTION

(1) *General considerations*

The phylum Protozoa may be divided into the subphyla Plasmodroma and Ciliophora. Included in the Plasmodroma are many forms frequently classified as Algae. The majority of these holophytic forms will be omitted from this review. There are, however, orders and even single genera in which some species are holophytic whereas others require complex food substances. The modifications of nutritional requirements from the holophytic to the saprozoic or holozoic types appear to be numerous and varied. Among the Ciliophora the range of nutritional types seems generally more restricted, but frequently the precise nutritional requirements are less readily determinable. Symbiotic and parasitic forms provide special cases.

In the history of the culture of a given protozoan from the time of isolation from its natural habitat to the time of definition of its nutritional requirements one may frequently distinguish five stages, characterized by the type of culture medium employed, viz.* (1) infusion or enrichment media; (2) biologically defined media—'species-pure culture', 'zweigliedrige' or 'pure mixed' cultures; (3) bacteriologically pure media; (4) chemically defined media; (5) biochemically defined media.

Infusions of hay, grains, garden earth, malted milk, meat extract, etc., in pond, spring or tap water, left open to bacterial inoculation, have furnished permanent laboratory sources of many organisms, but do not permit of the definition of the nutritional requirements. One step towards this definition occurs in the culture of the phagotrophic forms in 'species-pure' culture,† that is, the culture of a single species of protozoan on other known or definable micro-organisms. Pioneer work of this kind was done by Hargitt & Fray (1917) on *Paramecium*. The culture of an organism in the absence of all other organisms, viz. 'bacteriologically pure' culture, was achieved for a diffusive feeder, *Polytoma*, by Ogata in 1893, and for the ciliate *Glaucoma* by A. Lwoff in 1923. (For possibly prior pure liquid cultures see A. Lwoff, 1932.) Cultures of organisms on sterile infusions and decoctions are included as bacteriologically pure media.

The term chemically defined, as distinct from bacteriologically pure media, implies either the elimination of the metabolic products of other organisms or their chemical definition. For certain purposes media containing theoretically well-analysed peptones have been included among chemically definable media. The transition to chemically definable media is not always merely the problem of finding a fluid medium of suitable constitution; some phagotrophs require the presence of a particulate food. The effect of the viscosity of the liquid media may also be significant (Cosmovici, 1931 *a*, *b*, 1934 *a*, *b*; Mast & Doyle, 1934). Not only feeding reactions but also morphology and type of reproduction may be influenced by the nutrient medium (Kidder, 1941; Kidder & Stuart, 1939 *a*, *b*; Nattan-Larrier & Dufour, 1936; Stuart, Kidder & Griffen, 1939; and others).

The distinction between chemically and biochemically defined media is an artificial one made for this review to distinguish between the published recipe for the medium and the material in the culture flasks. The distinction is in principle a little more than a statement of the uncertainty of chemical constitution of media, for significant advances have now been made in the analysis of standard ingredients. A knowledge of expected traces of inorganic and organic substances deriving from particular brands of reagents, of ionic species differences in apparently identical formulae (e.g. the generally higher iron content of mixtures of sodium acetate and ammonium chloride than in sodium choride and ammonium acetate), of recently discovered chemical sources of contamination dependent on the technique of culture, and of evaluation of the food reserves carried within the organism, have all led to marked revisions of opinion concerning the apparent requirements of organisms. Hall (Calkins & Summers, 1940) has estimated that there are probably 100 bacteria-free strains of Protozoa now in existence.

From the point of view of rigid chemical definition of nutritional requirements we find that many of the papers published prior to M. Lwoff (1933 *a*) (trypanosomes), Hall & Elliot (1935), and A. Lwoff & Dusi (1937 *a*) (flagellates and ciliates) which deal

* Hall (Calkins & Summers, 1940) distinguishes four types of protozoan population: (1) the pure culture (bacteria free); (2) species-pure culture—a single protozoan species fed on other non-protozoan organisms; (3) mixed populations—two or more protozoan species; (4) wild populations.

† This step hardly applies to the culture of 'diffusive feeders', which term is intended to refer to holophytic and other forms as opposed to phagotrophs but to exclude those vacuole-forming forms which are normally phagotrophic but capable of growth in fluid media.

with the nature of the carbon and nitrogen energy sources utilizable by an organism disregard certain 'growth factors', especially vitamins (Hall, 1937*a*). This neglect may be ascribed to the fact that only recently have pure preparations of vitamins become available, and still more recently has it been realized that certain techniques (use of cotton plugs, *p*H and temperature during sterilization) have a marked effect on the vitamin content of the media. The dependence of Protozoa on these 'growth factors' is now in the process of active investigation, the requirements of different species being found to vary widely.

The definition of 'growth factors' is in some dispute (A. Lwoff & Dusi, 1937*a*; Schopfer, 1939). Some authors would include all substances which have a marked effect on growth in concentrations less than 10^{-10} *M*. We shall designate this range of concentration as that of the 'oligodynamic substances'. This includes the 'pseudo-growth factors' of inorganic nature (*Spurenelemente*), notably iron, manganese, zinc, copper, vanadium and molybdenum. Growth factors of the auxin type are also to be segregated from growth factors sensu stricto. Growth factors of vitamin-like nature which have been investigated (Schopfer, 1939; A. Lwoff, 1938*a*) include pyrimidines and thiazols (with groups substituted in various positions), thiamin (aneurine, vitamin B_1), riboflavin (lactoflavin, vitamin B_2), adermin (B_6), ascorbic acid (vitamin C), nicotinic acid, cozymase (factor V), hematin (factor X), pimelic acid, cholesterol (and over seventy related substituted sterols), uracil, inositol (Bios I), β-alanine (Bios II*a*) and others. These later studies profoundly change the interpretation of earlier work, consequently the early work will be reviewed somewhat independently. General treatments of the role of these oligodynamic substances in the nutrition of micro-organisms are to be found in Neipp (1937) (cations), Pirschle (1938) (*Spurenelemente*), and in Schopfer (1939) (vitamins and growth factors).

It is to be noted that the classification of a substance as oligodynamic cannot be based on concentration alone. If growth is likewise minute the substance may be an ordinary metabolite. Proof of oligodynamic nature requires that the relation between volume of growing organisms and concentration of active substance be in the ratio of substrate to enzyme concentration. There can, however, be no sharp distinction between substances contributing to enzyme formation and to intermediate carrier systems.

(2) *Methods*

(*a*) *Glassware.* The type of glass employed may influence the medium markedly. Changes in *p*H were observed by Esty & Cathcart (1921) to result from the use of certain types of glass. In isolation cultures on depression slides where the glass surface involved is large compared to the volume of the fluid marked toxicity of various glasses was observed by Raffel (1930).

(*b*) *Cotton plugs.* Schopfer & Rytz (1937) point out that the crude brownish cotton used for plugging culture vessels is a source of contamination, probably by pyramidine and thiazol, but that the white material is totally inactive. A. Lwoff & Dusi (1938*a*) have found a slow growth in cultures plugged with cotton in media which would not support growth in glass-stoppered vessels.

(*c*) *Sterilization and proof of sterility.* The usual bacteriological procedure for the preparation of sterile media has been applied to the culture of Protozoa with certain specializations. Many media (containing sugars or iron) require the separate sterilization of components. Sterilization of the organisms has been accomplished by various washing and dilution methods: Claff (1940), Kidder (Calkins & Summers, 1940), Kalmus (1931), A. Lwoff (1932), Parpart (1928), and Sawano (1938). In addition to the usual successive washings required for diffusive feeders phagotrophs require to be left in the washing solutions long enough to eliminate bacteria present in their food vacuoles. A. Lwoff (1932) emphasizes the necessity for proof of sterility by plating tests.

(*d*) *Estimation of nutritional adequacy of the medium.* In testing a simple medium for its ability to maintain growth, when the organism has originated directly from a more complex medium, the significance of the original medium transferred along with the organism has been taken into account by A. Lwoff and by Hall. A sufficient number of passages or subcultures is required to reduce the original inoculum to 10^{-10} *M* before it may be considered absent. For example, *Chilomonas paramecium* will grow on 5×10^{-6} *M* peptone, but not on lower concentrations (Hall, 1939*b*). Furthermore, sufficient new growth must occur to remove the oligodynamic constituents (contaminants) from the fresh medium. That the original medium transferred with the inoculum may be responsible for allelocatalytic effects and for biological conditioning of the medium has been shown by Hall (1941*a*) and Hall & Loefer (1940).

In cases where repeated transfers show diminution and ultimate disappearance of dense growth one may rightly conclude that the medium is inadequate for the particular strain. However, during transfer through a medium inadequate for the large majority of the organisms some individuals (a new strain) may start a very slow growth (Hall & Schoenborn, 1939*c*). Unless cultures are retained for fairly long periods the newly developing strain may be overlooked. Such differences may account for discrepancies in the literature in cases where strains have not been interchanged between investigators. Isolation culture methods involve per unit volume of protozoan a very much greater potential source of contamination which may be adequate to supply not

only all required oligodynamic substances but also essential metabolites.

Tests for the dispensability of any substance or any ion require a degree of purification of the medium which may be approached in various ways. The substance may be removable by heating (X substance of Mast & Pace, 1938 *b*, *c*). The possibility of absolute elimination of certain ions from media by purely chemical procedure is in many cases impracticable. Glass, air, gas flames, original or repurified chemicals, frequently retain or introduce traces by no means insignificant. Providing that adequate growth may be attained in a given amount of medium exposed to limited contamination by glass, etc., the organisms will remove the traces of required substances from the medium; large glass and air surfaces (e.g. of the isolation culture technique) make this approach impossible. When the original medium is definable and the bacteriological purity of the organisms comprising the inoculum has been established, the adequacy of the medium may be judged by various criteria: (1) whether the organism will grow indefinitely with requisite transfers (A. Lwoff, 1932; Hall, 1939*b*), (2) whether the rate at which an organism grows in a given medium is maintained, accelerated or depressed in reference to a medium known to be adequate.

An analysis of growth is beyond the scope of this review. A number of reviews on the significance of population curves are available (Beers, 1933 *a*, *b*; Brown, 1940; Buchanan & Fulmer, 1928; Fauré-Fremiet, 1922; Gause, 1934 *a*, *b*; Hall & Shottenfeld, 1941; Hutchens, 1941; Jahn, 1934*b*, and others ibid.; Ludwig & Boost, 1939; Park, 1939; Rahn, 1932; Richards, 1932; and Volterra, 1931, 1934). Distinction between growth in volume and growth in number of organisms has been dealt with by Adolph (1931), Ferber, (1928), Harding (1937 *a*, *b*), Loefer (1938*b*), Zingher & Fisikow (1931), and Zingher, Narbutt & Zingher (1932). Changes in structure, size, mode of reproduction, effects of training, selection of genetic types, the development of adaptive enzymes, etc. (Jennings, 1929; Knight, 1936) have also to be considered.

An outline of the degree of control required before one may profitably proceed to mathematical analysis of growth has been given by Jahn (1934*b*). As factors influencing growth he lists changes in: (1) quantity and quality of available food or food organisms, (2) concentration of waste products, (3) *p*H, (4) oxygen and carbon dioxide tension, (5) temperature, (6) light, (7) when bacteria are present, numerous specific compounds produced by bacterial action. He further discusses the possible physiological differences between species and the role of oxidation-reduction potential. Allelocatalyses are dealt with below. Counting methods employed include dilution and direct counting, the use of special counting chambers, etc. (Hall, Johnson & Loefer, 1935; Harding, 1937*a*). Methods of calculation are given by Loefer (1938*a*) and A. Lwoff (1935*a*). The use of parallel control cultures (Hall & Schoenborn, 1939*b*) gives rise to the variations pointed out by Kidder (1941) who employs special means for direct aseptic sampling.

II. PHYSIOLOGICAL CLASSIFICATIONS

A terminology and a system of classification adequate for the existing nutritional types of Protozoa has yet to be developed. Bases for an adequate terminology have been examined by A. Lwoff (1932) and Pringsheim (1937*d*). The older terms holophytic, saprophytic, parasitic and holozoic are too diffuse, and between these terms there exist intermediary types (Pringsheim, 1937*d*). Nutritional classifications of the type originated by Pfeffer in 1897 have been proposed by a number of investigators. A. Lwoff (1932) adopts the classification of Chatton, and divides the eucaryote Protista into (*a*) the Protozoa sensu stricto (hereafter abbreviated as 'Protozoa s.s. Lw.'), viz. organisms containing a nucleus and mitochondria but lacking chlorophyll, (*b*) the chlorophytes, viz. eucaryote organisms with one or more plastids containing chlorophyll, and (*c*) the leucophytes which have one or more plastids but no chlorophyll.

A. Lwoff (1932, and as modified later) presented a terminology based primarily on the nitrogen and carbon requirements of the organism. Prototrophic nutrition (Fischer) is the nutrition of organisms assimilating molecular nitrogen. Autotrophs (Pfeffer) are organisms capable of synthesizing all required nitrogen and carbon compounds from inorganic sources. The energy required is furnished either by oxidation of inorganic compounds, e.g. sulphur or iron: chemoautotrophy, or, as a result of photosynthesis: photoautotrophy. Thus organisms utilizing inorganic nitrogen sources by means of energy supplied by organic carbon compounds are excluded from the class autotrophs. Mesotrophs are organisms capable of synthesizing their required nitrogen compounds either from ammonia in the presence of an organic compound or from the nitrogen of a simple organic compound (e.g. an amino acid). If the source of ammonia is inorganic an organic nitrogen source is required. (Such nutrition is not synonymous with mesotrophy as defined by Naumann (1932) or Pringsheim (1937*d*).) If the source of nitrogen be organic it may serve as the source of carbon as well: haplotrophy. A. Lwoff (1938*a*) states that haplotrophs and Protozoa s.s. Lw. although capable of utilizing fatty acids develop perfectly without them (but see Pringsheim, 1937*d*). The source of the nitrogen may be either inorganic or from a simple organic substance, but an additional source of carbon is required: oxytrophy. 'La multiplication de tous ces organismes (meso- et metaoxytrophs)—est considérément ameliorée par les

acides gras ou certains autres acides organiques' (A. Lwoff, 1938*a*). The independent carbon source may be required to arise from a photosynthetic process: obligative phototrophy. If the source of nitrogen is a simple compound: mesotrophy. Metatrophs are organisms incapable of effecting synthesis from simple nitrogenous substances; a complex (e.g. peptone) source is required. Metatrophy, like mesotrophy, may be accompanied by carbon nutrition which is photo-, oxy-, or haplo- in nature. Haplometatrophy is difficult to determine, since in a medium containing so complex a source of nitrogen as peptone it is not possible to know whether the compound furnishing the nitrogen is also furnishing the carbon (Pringsheim, 1937*d*).

A. Lwoff (1938*a*) states that all oxytrophs studied to date correspond to the 'Fettesäure Organismen' (acetate organisms) of Pringsheim (1937 *a*, *b*, *c*, *d*). Pringsheim (1937*d*) states: 'Ich stelle den Begriff Azetatorganismen dem seinen der Oxytrophen gegenüber und verstehe darunter solche Lebewesen welche durch Verarbeitung neideren Fettsäuren, vor allem Essigsäure, in ihrer Entwicklung stark gefordert werden, während Zucker, Alkohole und andere organische Verbindungen weniger geeignet sind.' A. Lwoff (1935*a*, 1938*a*) has modified the term oxytrophy. He states (1938*a*, p. 205): 'L'oxytrophie au sens large sera ainsi definie: condition physiologique des organismes auxquels un aliment carboné organique independent est indispensible, en plus de l'aliment azoté organique, ou sur le development desquels les aliments carbonés exercent une action favorisante spécifique.' 'L'oxytrophie est liée à la synthèse des réserves glucidiques figurées en l'absence de...photosynthèse.' In A. Lwoff (1938*a*) and in A. Lwoff & Dusi (1935*a*) is the statement 'la synthèse des réserves glucidiques figurées est liée à la présence d'une plaste'. A. Lwoff (1935*a*) also attempted to establish a measure of oxytrophy which he termed the oxytrophic coefficient. This is defined as the ratio of the maximum number of organisms developing in a medium containing acetate to the number developing in the same medium deprived of acetate. He found coefficients of 1000 for *Polytoma uvella*, 40 for *Astasia chattonii* and values less than 1 for Protozoa s.s. Lw. (since in most cases the fatty acids were found to inhibit development). He found, however, wide variation in the values for different cultures.

Pringsheim (1937*d*) criticizes at some length the entire classification of Lwoff and the terminology of Chatton used by Lwoff. The greatest difficulty is experienced with the terms oxytrophy and haplometatrophy and with the group leucophytes. Pringsheim points out that an ideal classification must approach the subject not only from purely biochemical principles but also in consideration of ecological factors. He proposes that the basis of Lwoff's classification should be modified somewhat, the distinction between oxytrophy and haplometatrophy being eliminated. Pringsheim maintains that the term oxytrophy as used by Lwoff is synonymous with heterotrophy (Pringsheim), and that the existence of haplotrophy has not been established.

Hall (1939*b*) proposes a simplified classification which likewise includes oxytrophs and haplotrophs within the term heterotroph (Pringsheim). Hall uses the term autotroph for all organisms utilizing inorganic nitrogen regardless of the nature of the carbon source, whereas Lwoff retains Pfeffer's usage so that an organism must be able to utilize inorganic carbon to be considered autotrophic. The systems are compared in Table 1. The distinction between meso- and metatrophs is made on the basis of the nitrogen requirements of the organisms; but in the substitution of peptone media for simpler nitrogen compounds vitamins and other accessory substances may be introduced into the complex medium (Hall & Elliot, 1935; Elliot, 1939; Hall, 1942). Nevertheless, the chemical analyses of media in which *Acanthamoeba* and *Glaucoma* have been grown indicate the use of protein and polypeptide nitrogen respectively.

At the moment of writing it is not possible to evaluate the terminology definitively. It might seem inadvisable to employ terms so poorly definable. However, the need for a terminology is so great, and any other descriptive method so diffuse, that the terms used by the original authors will be given here followed by the symbols (Lw.) for Lwoff, (Pr.) for Pringsheim, and (Hl.) for Hall. In some cases a given organism may be maintained in more than one nutritional manner: the nutritional classification of an organism is here defined as that of the least complex culture condition adequate for maintenance.

III. NUTRITION OF REPRESENTATIVE ORGANISMS

(1) *Polytoma* and *Polytomella*

Polytoma uvella (Ehrenberg) is a colourless phytomonad of the family Chlamydamonadidae (Bütschli). Its nutritional requirements are similar to those of *Polytomella* of the family Carteriidae. *Polytoma* was studied by Pascher (A. Lwoff & Provasoli, 1937), and various subdivisions of the species *P. uvella* noted. Of these *P. uvella obtusum* has been found to have nutritional requirements differing from *P. uvella*, and in later work (A. Lwoff & Provasoli, 1937) it is designated *P. obtusum*. A. Lwoff (1935*a*, *b*) and Pringsheim (1935) refer to *Polytomella agilis* which in later work was found to be *P. caeca*.

Ogata in 1893 seems to have been the first to obtain the bacteriologically pure culture of a leucophyte (Lw.) on a complex medium containing bouillon, glucose and the decoction of an alga. Pringsheim (1921) arrived at simple media suitable to growth of *Polytoma*. These solutions contained: solution I, sodium acetate, glycine, glucose, potassium car-

bonate, magnesium sulphate, dipotassium phosphate; solution II, magnesium sulphate, dipotassium phosphate and ammonium acetate. Pringsheim arrived at these formulae by an interesting series of theoretical deductions. Doflein (1916, 1919) had previously established the group 'Zuckerflagellaten' including *Polytomella*. According to Doflein this group requires sugar in soluble form in the medium, but he made no inquiry into bacteriological purity (see A. Lwoff, 1932). Pringsheim (1921) found that ammonium acetate (solution II) was as suitable a source of carbon and nitrogen as glycine plus sodium acetate and sugar (solution I). Various sugars and starch were found to have very little accelerating effect on growth if acetate was present, and if acetate was absent very feeble growth occurred with the sugars present. He found that formic acid could not replace acetic in solution I; butyric gave poor cultures, but was used; proprionic, oxalic, citric, malic, tartaric and succinic acids were useless. In the same medium (I) he tested the growth when glycine was replaced by alanine, leucine, aspartic acid, tyrosine, peptone and egg albumin. All gave good cultures but none so good as glycine. Aspartic acid was best, albumin worst, and peptone not much better than albumin. Nitrates were useless. It was later found by A. Lwoff & Dusi (1938*a*) that *Polytoma ocellatum* alone (of other *Polytoma* and of *Polytomella* species) is capable of using potassium nitrate as a source of nitrogen if carbon is supplied by ethyl alcohol or acetic acid.

Table 1. *Physiological classification of micro-organisms*

	Chemotrophs	Phototrophs	Heterotrophs	
	Use CO_2 by energy from oxidation of inorganic substances	Chlorophyll-bearing spp.; light energy and CO_2 used in photosynthesis; some appear to be obligate phototrophs	Colourless. Organic C source necessary in absence of chlorophyll. Some chlorophyll-bearing spp. can grow in darkness, thus facultative heterotrophs	
			Oxytrophs: Whatever the N source, independent organic C source	Haplotrophs: Organic N source can serve as C source
Metatrophs: Complex N source (peptone)	Chemometatrophs: ?	Photometatrophs: Grow in peptone but not amino acid or inorganic solutions, e.g. *Euglena pisciformis*	Heterometatrophs: Grow in peptone but not amino acid or inorganic solutions, e.g. *Hyalogonium Klebsii*	
			Oxymetatrophs	Haplometatrophs
Mesotrophs: Protein synthesized from N of ammonia, nitrate, amino acid or more complex organic N comps. only when organic comp. present	Chemomesotrophs: ?	Photomesotrophs: Grow in media containing amino acids but not inorganic, e.g. *Euglena deses*	Heteromesotrophs: One or more amino acids as sources of N and C; usually more growth with additional C source, e.g. *Polytomella caeca*, *Astaria quartana*	
			Oxymesotrophs	Haplomesotrophs
Autotrophs: N and C sources both inorganic	Chemoautotrophs: Bacteria growing in inorganic media without using light energy, e.g. *Beggiotoa*, unknown in Protozoa	Photoautotrophs: Grow in inorganic solutions, e.g. *Chlorogonium euchlorum*, *Euglena gracilis*	Heteroautotrophs: Use inorganic N comps. with organic C source, e.g. *Polytoma uvella*	
Prototrophs: Assimilate molecular N	Chemoprototrophs: ?	Photoprototrophs: Cyanophyceae?	Heteroprototrophs: Azotobacter	

Pringsheim & Mainx (1926) made a systematic survey of the relation of the structure of the fatty acids to their suitability as carbon sources, and found that those aliphatic acids with an odd number of carbon atoms were less favourable for growth than those with an even number (those occurring naturally). A. Lwoff (1929, 1931, 1932) reinvestigated this

with reference to the normal and iso-configurations, and concluded that the number of CH_2 groups is more significant than the total number of carbon atoms. (But see Barker (1935), who used other methods.)

Volkonsky (1930 *a*, *b*, *c*) (*Polytoma uvella*) and A. Lwoff & Provasoli (1935) (*Polytoma caudatum* var. *astigmata*) made cytological studies and described the reticulate plastid. Volkonsky noted that the volume of the leucoplast varied with the source of nitrogen, being maximal in the simplest media (e.g. ammonium acetate) and very attenuated in peptone (see also A. Lwoff, 1932). Pringsheim had found poor growth on peptone and Lwoff reinvestigated the adequacy of such media. He found (1932) that ereptone, silk peptone, tryptic digests of beef, peptic digests of beef and of peanut, and yeast autolysate were all adequate sources of nitrogen. Pringsheim (1937 *c*) also investigated particular peptones with *Polytoma uvella* and *Polytomella caeca*.

In the work of Hall (1939 *b*), A. Lwoff (1929, 1931, 1935 *a*, 1938 *a*), A. Lwoff & Dusi (1938 *a*), Pringsheim (1937 *c*), Provasoli (1937 *a*, *b*) and others traces of 'Spurenelemente' were generally assumed to be present. The presence of traces of organic nitrogen results in the appearance of a bios effect (Wildiers), but this disappears on the addition of traces of iron. The relation of oxygenation of the media to growth is presented by Rottier (1936).

Prior to the establishment of the role of vitamin B_1 (thiamine, aneurine) in the nutrition of these leucophytes (Lw.) and before the significance of the growth factors present in peptones (Elliot, 1939; Hall, 1942; M. Lwoff, 1937; and others) and in cotton used for plugs (Schopfer & Rytz, 1937) was realized, much was written on the comparative nitrogen and carbon requirements of *Polytoma* and *Polytomella*. In addition to those referred to above there were papers by A. Lwoff (1935 *a*, 1938 *a*), A. Lwoff & Dusi (1934), A. Lwoff & Provasoli (1935, 1937), Pringsheim (1927, 1935, 1937 *d*, *e*), Provasoli (1937 *a*, *b*).

The universal suitability of most peptone media has been discussed above. A. Lwoff (1929, 1931, 1932) and Pringsheim (1921, 1937 *c*) find that *Polytoma uvella* is capable of heteroautotrophic (Hl.) nutrition in ammonium acetate media. The acceleration of growth by addition of fatty acids (in the presence of an adequate source of nitrogen) has been studied for *Polytoma* spp. and *Polytomella* spp., and species variations noted (Hall, 1939 *b*). All are capable of heteromesotrophic (Hl.) nutrition (Hall, 1939 *b*). In the work on *Polytoma uvella* (A. Lwoff, 1929, 1932; Pringsheim, 1921; Pringsheim & Mainx, 1926), *P. caudatum* (A. Lwoff & Provasoli, 1935), *P. ocellatum* (Pringsheim, 1937 *a*, *c*, *d*), *Polytomella caeca* (Pringsheim, 1935; A. Lwoff, 1935 *b*), multiplication was found to be considerably increased by fatty acids and certain other organic acids (Lwoff, 1938 *a*). The findings of Pringsheim up to 1937 are at variance with those of A. Lwoff & Provasoli (1937) and Provasoli (1937 *a*, *b*). Provasoli attributes the differences to the fact that Pringsheim may have used concentrations of certain fatty acids which were too high. A. Lwoff & Provasoli (1937) found that *Polytoma obtusum* differs from *P. uvella* in that whereas both use acetic and butyric, *P. obtusum* cannot use proprionic, isobutyric, valerianic and caproic, while *P. uvella* s.s. Lw. can use acetic, normal butyric, normal valerianic, normal caproic, and isocaproic, but not any proprionic, isobutyric, or isovalerianic.

The role of thiamin (aneurine, vitamin B_1) and of its pyrimidine and thiazol constituents as growth factors for *Polytoma* and *Polytomella* was studied by A. Lwoff & Dusi (1937 *a*, *b*, *c*), but their findings were later modified. Lwoff (1938 *b*), in a general review of growth factors for Protozoa and related organisms, states that *Polytomella caeca* and *Chilomonas paramecium* require both of the constituents of thiamin, *Polytoma ocellatum* is capable of synthezing the pyrimidine it needs but must have the thiazol supplied, whereas *P. obtusum* and *P. uvella* can synthesize the thiazol but require the pyrimidine to be supplied. All of the above forms are independent of thiamin if both constituents are supplied. A. Lwoff & Dusi (1938 *a*, *b*, *c*) examined the effects of substitution of various groups in the pyrimidine and thiazol fractions and found that six of the thiazols were suitable for growth (1938 *c*), and that these organisms were capable of detaching pyrimidine and thiazol from molecules unsuitable as growth factors in themselves. In general they found (1938 *b*) that the substances active for *Staphylococcus aureus* and for *Phycomyces* were suitable growth factors for *Polytoma*, *Polytomella* and *Chilomonas*, and substances inactive for the former were inactive for all.

(2) *Chilomonas paramecium*

Chilomonas paramecium is a colourless cryptomonad. Structural studies on its nutrition have been made by Bütschli (1868 in A. Lwoff, 1938 *a*), Fisch (1885), Hall (1930), Mast & Doyle (1935 *a*), Mast & Pace (1933 et seq.), Gatenby & Smyth (1940). Leucoplasts are present and starch is usually prominent. The nutritional requirements are in some dispute. Mast maintains that his strain and that of Hall and Loefer can be grown on acetate and ammonium solutions in depression slides and in mass cultures. The solutions used were those of Mast & Pace (1933) with subsequent modifications (Mast & Pace, 1938 *c*; Burrows, 1938; and Bowen, 1940). Acetate may be replaced by carbon dioxide if silicate is present (Mast & Pace, 1933, 1937; Burrows, 1938). Urea and formate are suitable sources of carbon, but less favourable than acetate (Mast & Pace, 1933). For the utilization of acetic, proprionic, *n*-butyric, *n*-valerianic, *n*-caproic, isobutyric, ethyl acetate, malic, lactic, pyruvic and succinic acids, ethyl alcohol, glyceric aldehyde and dihydroxyacetone in

the presence of 'peptone de viande Vaillant' by *Chilomonas*, Pringsheim (1937*a*) found that of ten species studied sodium acetate was the substrate preferred by all except *C. oblonga* which grows better on succinate. Ethyl acetate is utilized by all. The importance of the concentration is pointed out by Mast & Pace (1933). The density of growth attained is limited by the excretion of a substance 'X' (Mast & Pace, 1938*c*) which is an accelerator of growth and reproduction in optimum and suboptimal and an inhibitor in supraoptimal concentrations. (See also Hall, 1941 *a*, *b*, and Kidder 1941.)

Mast & Pace (1933) state that when *C. paramecium* grows in their inorganic solution, ammonium chloride is oxidized to nitrates, that the energy used in synthesis (of protoplasm and reserves) is largely, if not entirely, obtained from this oxidation, and that light has no effect on synthesis. This places *C. paramecium* in the unique position of being capable of chemoautotrophic nutrition (Lw.). The formation of nitrate could not be confirmed by Burrows (1938), nor could bacterial contamination be found. Hall & Loefer (1936), Mast & Pace (1933) and Pringsheim (1921) are in agreement that *C. paramecium* is capable of oxymesotrophy (Lw.) (heteromesotrophy, Hl.) in solutions of glycine and acetate. Heteroautotrophic nutrition in solutions of ammonium acetate has been denied by Loefer (1934), Loefer & Hall (1936), A. Lwoff (1935*a*), Pringsheim (1936), and the difficulties of refutation are discussed by Hall (1939*b*). The role of selection is discussed by Hall & Schoenborn (1939 *a*, *b*). If, however, thiamin or its constituents are present in the medium, *Chilomonas* is capable of growth on ammonium acetate (A. Lwoff & Dusi, 1937*b* et seq.). Hutchens (1940, 1941) finds utilization of ammonia and of marked amounts of acetate when vitamin B_1 is added, the utilization of material being beyond the limit provided by food traces and in excess of the added B_1.

A series of studies prior to those of A. Lwoff & Dusi (1937*b*) concerning the comparative suitability of various carbon and nitrogen sources for the nutrition of *C. paramecium* was made by Loefer (1935*a*, 1938*a*) (sugars), Loefer (1933, 1935*b*), A. Lwoff & Dusi (1934), Pringsheim (1935, 1937*a*), Provasoli (1937*b*) (fatty acids), Loefer & Hall (1936), Oliphant (1939) (alcohols), Loefer (1932, 1935*b*), Pringsheim (1937*a*) (peptone and fatty acids). Loefer (1937, 1938*a*) could find no quantitative evidence of the fermentation of carbohydrates nor any evidence of appreciable utilization of glucose.

In general, the studies on the relative accelerations of growth produced by various fatty acids conform with those of other oxytrophs (Lw.) or acetate organisms (Pr.). Loefer (1933, 1935*b*) found that acetate, butyrate, valerate, proprionate accelerate growth. No acceleration was found with ethyl alcohol by Loefer & Hall (1936). Provasoli (1937*b*) found that *C. paramecium* could use isovalerianic, caproic, isocaproic, heptylic, and octylic acids.

Barker (1935) found that *Prototheca Zopfii* cannot develop anaerobically, but that if a large number of cells grown aerobically is added to a strongly buffered (calcium carbonate) solution of glucose, the glucose is converted rapidly and almost quantitatively to lactic acid. He further found that although ammonium chloride is assimilated, even in the presence of a suitable carbon source an organic nitrogen source more complex than amino acids is required. Yeast autolysate or other source of complex organic material is required, but whether this is a source of nitrogen or accessory substances is not certain. By quantitative manometric experiments, of brief duration as compared to culture methods, Barker established that all saturated fatty acids tested (up to palmitic) are utilized except formic and possibly isovalerianic. Both the straight (*n*-) carbon chains and the branched (iso-) chains are oxidized, but the latter less readily. No preference was found for the naturally occurring fatty acids (those with an even number of carbon atoms). No hydroxy, keto, phenyl-substituted or dicarboxylic acid was found to provide a carbon source. *Prototheca* attacks monoalcohols and ketones, also dihydroxyacetone and glycerol, but not glycol, erythritol and mannitol. Quantitative analyses of the cells showed that large percentages of the substrates had been converted to cell material. The work of Loefer (1937, 1938*a*), using culture methods to determine carbon utilization for *Chilomonas*, need not necessarily rule out fermentation and utilization of other carbon sources under conditions such as those of Barker for *Prototheca*. Fermentation or glycolysis of substrates or food reserves may take place for relatively brief periods without providing adequate nutrition.

Pringsheim (1937*a*) investigated the growth of *Polytoma uvella* and other organisms in media containing 'peptone de viande (Vaillant)' to which a fatty acid or related substance was added. He found that acetic acid and ethyl acetate were favourable to the growth of all forms, and that these substances were preferred by all but *Chilomonas oblonga* which preferred succinic acid (see also Provasoli, 1937*b*). Loefer (1932, 1935*b*) found that good growth of *Chilomonas paramecium* occurred on peptone of high amino-nitrogen content and that slow growth occurred on unhydrolysed protein, casein and gelatin. Pringsheim (1937*a*) also reported good growth on peptone.

(3) *Euglena*

This genus has been extensively studied. Reviews of the literature have been made by Hall (1939*b*, 1941*b*, in Calkins & Summers) and A. Lwoff (1932, 1938*a*). Conclusions of these reviewers, expressed in their own nomenclature, emphasize the need for an adequate terminology and more precise definition of experimental conditions. Thus from almost

identical literature, Hall (1939*b*) concludes that *Euglena gracilis* is a facultative autotroph (Hl.) capable of photomesotrophy (Hl.), whereas Lwoff (1938*a*) concludes that it is an oxymetatroph (Lw.). The question of autotrophy (Hl.) in *E. gracilis* has been studied by Dusi (1930 *a*, *b*, *c*, *d*, 1933*a*), Hall (1939*a*), Hall & Schoenborn (1939*a*), Hutner (1936), A. Lwoff (1932, 1938*a*), A. Lwoff & Dusi (1929, 1931), Mainx (1928 *a*, *b*), and Pringsheim (1912, 1937*d*). The conflicting results are to be explained in part by unsuitable inorganic media and concentration of the food (Hall, 1939*b*). Acceleration of growth by acetate and other suitable carbon compounds (oxytrophy, Lw.) has been reported by Dusi (1933*a*), Hall (1939*a*), Jahn (1935), Loefer & Hall (1936), A. Lwoff (1932), Provasoli (1938*b*), and Ternetz (1912).

E. gracilis may be grown in the dark as a heteromesotroph (Hall, 1939*b*). In darkness growth is not accelerated by plant-growth hormones, whereas in the light it is (Elliot, 1937*b*). The acceleration of growth produced by butyrate, acetate and lactate is greater in darkness than in light (Jahn, 1935). In darkness sodium succinate accelerates growth: in light it is toxic (Jahn, 1935). *E. stellata* responds to increased concentrations of acetate by decreased growth in light and increased growth in darkness (Hall, 1937*b*). Marked acceleration of growth in peptone media by sodium acetate has been recorded for several species (Hall, 1939*b*), and the question of phototrophic nutrition is reviewed in this paper. Other species were found to be questionable photoautotrophs. Mainx (1928 *a*, *b*) found all the forms investigated by him to be autotrophs (Hl.). Dusi found that *E. anabena* (1933*b*) is photoautotrophic and that *E. pisciformis* (1933*b*), *E. viridis* (1936) and *E. deses* are not. Hall concludes that *E. anabena* (1938) and *E. viridis* (1939 *a*, *b*, 1941*b*) are photoautotrophs. Dusi (1933*b*) concludes that *E. deses* is an obligate photomesotroph (Hl.) and that *E. pisciformis* is an obligate photometatroph. Chlorophyll-bearing forms have been cultured in the absence of light, and certain of them have been found to be heterometatrophic in darkness. Others require light and so are obligate phototrophs. Some are obligate phototrophs on certain media but not on others. Hall (1939*b*) gives a list of species in the different categories. Photometatrophy can be carried on by all chlorophyll-bearing flagellates which have been established in pure culture.

Hall & Schoenborn (1939*b*) point out that upon transfer of *Euglena* from peptone to inorganic media there may occur a selection of a comparatively small proportion of facultative photoautotrophs while the rest of the population dies. Such a process may account for some of the contradictions in the literature where the consequent 'long lag phase' with no apparent growth may have led some investigators to conclude that growth was not taking place. The possible effects of various vitamins on the obligate nature of the phototrophy have yet to be examined. Dusi (1939) reported that *E. pisciformis* requires thiamin even in light, but this is questioned by Hall (Calkins & Summers, 1940).

Astasia Chattonii (*A. longa*), *A. quartana* (*A. ocellata*), and *Khawkinea halli* (Elliot, 1938) are all oxytrophs (A. Lwoff, 1938*a*). The amount of growth acceleration produced by fatty acids varies with species (A. Lwoff & Dusi, 1934, 1936; Pringsheim, 1937 *a*, *d*; Provasoli, 1938*a*). They will not grow on single or mixed amino acids (Mainx, 1928*b*), but will grow on peptone, and Hall classifies them as uncertain heteromesotrophs (Hall, 1939*b*). Elliot (1938) found that no acceleration was produced by various plant hormones in *Khawkinea halli* (*Astasia* sp.) or in *Euglena gracilis* in the dark, whereas marked acceleration of growth in light occurred in *E. gracilis* (Elliot, 1937*b*). *Hyalogonium Klebsii* has been grown on various peptones (Hall, 1939*b*; Pringsheim, 1937 *a*, *d*), and its growth is accelerated by fatty acids (Pringsheim, 1937*a*, *d*). A. Lwoff (1938*a*) classifies it as an oxymetatroph. *Haematococcus pluvialis* is an oxytroph (Lw.) in darkness, and a photoautotroph (Hl.) in light (A. Lwoff, 1932). In peptone its growth is accelerated by acetate (Hall, 1939*b*) or other suitable carbon compounds (A. Lwoff 1932).

The growth of a number of species of *Chlamydomonas* is accelerated by fatty acids, especially acetate (Hall, 1939*b*; A. Lwoff, 1938*a*). In *Chlorogonium euchlorum* and *C. elongatum* there is a marked acceleration by various sugars (Loefer, 1933, 1935*a*). In peptone there is a marked acceleration by sodium acetate and other fatty acids (Hall, 1939*b*; Hall & Schoenborn, 1938; Loefer, 1933, 1934, 1935 *a*, *b*; A. Lwoff & Dusi, 1935*b*; Pringsheim, 1934, 1935, 1937 *a*, *b*, *d*). Both species are classed as oxytrophs by A. Lwoff (1938*a*). Loefer (1934) found that peptones with high amino-nitrogen content were more suitable than casein or gelatin. In darkness these species are heterotrophic, in light photoautotrophic, capable of obtaining nitrogen from ammonium nitrate (Loefer, 1934).

(4) *The ionic medium*

The ionic medium, as distinct from the availability of adequate carbon and nitrogen, has marked effects on the growth of Protozoa. Mast & Pace (1935) have studied the effects of sulphur in various forms on the growth of *Chilomonas*. Chilomonads starved in a medium lacking acetate and sulphur die in four days full of fat globules. If acetate is absent but sulphur present they die after six days, but fat globules are not prominent. In view of the results of Hammett (1930) and Jahn (1934 *a*, *b*) on the role of the sulphhydryl radical, Mast & Pace (1935) tested (in isolation culture) the following forms of sulphur: sodium sulphate, sodium sulphite, sodium sulphide, glutathione (oxidized and reduced),

cysteine and cystine. All were found to be suitable substitutes for magnesium sulphate, but the optimum concentration varied widely (see also Jahn, 1936 *a, b, c, d*). Mast & Pace (1937) conclude that silicon behaves as a catalyst. In the studies of Mast & Pace, and of Bowen, the effects of ions on the visible starch and fat content of the organisms is described, as well as on frequency of division in isolation cultures. On the relation between body size, metabolic rate and division rate see Adolph (1931). Using standard ammonium acetate medium (Bowen, 1940) for *Chilomonas* the effects on division rate and cytology of variations in calcium and magnesium (Mast & Pace, 1939), vanadium, copper, manganese, and iron (Bowen, 1938, 1939, 1940) were studied. Hall (1937 *c*) considered the effect of manganese on the growth of several organisms. Adaptation to latex media differing widely from the normal milieu for *Trypanosoma lewisii* has been recorded by Pannier (1936).

The work of Hall (1937 *b*, 1938), Hall & Schoenborn (1938) and Loefer (1939) shows the necessity for appropriate inorganic media, and thus accounts for the failure of Dusi (1930, 1931, 1936, 1939), Hall (1938) and Hutner (1936) to obtain growth of *Euglena* spp. The effects of different concentrations of sodium acetate on the growth of *E. stellata* are given by Hall (1937 *b*) (see also Hall, 1939 *b* and Hall & Schoenborn, 1939 *c*).

As regards hydrogen-ion concentration, the 'hay infusion cycle' is characterized by changes in *p*H correlated with the sequence of organisms. The relation was first clearly shown by Darby (1929, 1930 *a*). Similar studies have been made on *p*H tolerance and sequence of forms by Chejfec (1933), Date (1931), Eisenberg-Hamburg (1932), Fine (1912), Pruthi (1926), Ulehla (1923), and Woodruff (1912 *a*). Under controlled culture conditions the *p*H range for growth is more significant. Loefer (1935 *c*) found that for *Chilomonas* the curve of growth plotted against *p*H showed two maxima, one at *p*H 4·6–5·1 and another at *p*H 6·6–7·0, and that growth was greatest at *p*H 5·0. When acetate is added to tryptone media the peak at *p*H 5·0 disappears and maximal growth occurs near neutrality. *Chlorogonium euchlorum* and *C. elongatum* showed single maxima at *p*H 7·5 and 7·9 respectively. The *p*H range of growth in other forms is given by Loefer (1935 *c*).

(5) *Normally phagotrophic forms*

In forms normally phagotrophic (food vacuole formers) the determination of the adequacy of a medium for growth is dependent not only on the chemical constitution of the food but in some cases is complicated by selective ingestion (Bozler, 1924; Bragg, 1936; Kalmus, 1931; Losina-Losinsky, 1931; Lund, 1914 *a, b*). The effects of the physical consistency of the food medium have been studied by Cosmovici (1931 *a, b*) and Mast & Doyle (1934). The effects of temperature and ions on ingestion by *Amoeba* have been studied by Mast & Fennel (1938).

Early work on senescence and rejuvenescence in Protozoa brought to the fore the possibility that the nutritional adequacy of the medium was not the only factor in the maintenance of pure line cultures (Jennings, 1929; Jollos, 1921; Woodruff, 1911 *a, b*, 1912 *a, b*; Woodruff & Baitsell, 1911). More recent work by Jennings, Sonneborn, Wichtermann and others on genetic and sexual processes in *Paramecium* account for periodic fluctuations in rate of reproduction. Zweibaum (1922) studied the effect of conjugation on food reserve.

According to Furgason (1941) and A. Lwoff (1932), Oehler (1919, 1920, 1924) was the first to report the successful culture of ciliates in the absence of living bacteria. The successful liquid (non-particulate) culture of Protozoa has been most frequently achieved with various species of *Glaucoma* and *Colpidium*. These forms are so closely allied morphologically that much confusion in naming exists in the literature. For a critical review of synonomy and distinction between species see Furgason (1941).

A. Lwoff states that various parasitic flagellates are phagotrophs (1932). *Conidophrys* (Pilsuctoridae), which is enclosed in an envelope, ingests no food particles and forms no food vacuoles (Chatton & Lwoff, 1936). A historical review of the development of pure liquid culture from bacteriologically pure particulate media and from pure mixed cultures is given by A. Lwoff (1932, 1938 *a*). The technique of isolation, washing and the pure culture media employed by Lwoff and co-workers are reviewed by A. Lwoff (1932) and others cited above. Johnson (1941) and A. Lwoff (1938 *a*) list the normally holozoic forms which have been grown in pure culture.

Andrejev (1928) ascribed the acceleration of ciliate growth produced by garden earth extracts to the production of optimal conditions in hay infusions by its buffering action and its ability to adsorb noxious substances.

A. Lwoff (1938 *a*) reported the following as obligatory phagotrophs: *Vahlkampfia magna*, *Colpidium campylum*, *Bodo* sp., *Prowazekia* sp., *Colpoda steini* and *C. cucullus*. These have been grown in pure cultures of single bacteria, on dead bacteria, or on protein particles. Lwoff considers these forms haplotrophs (multiplication independent of the presence of free fatty acids).

Lilly (1942) found that *Stylonychia pustulata* and *Pleurotricha lanceolata* are carnivorous in that they cannot be cultured on preparations of the ciliates normally eaten unless these are alive. They can be grown on pure cultures on *Tetrahymena* and *Colpidium* plus a supplementary growth factor obtained from yeast or other plant material. The factor could not be identified with any known vitamin. Doyle & Patterson (1942) found that *Didinium nasutum*

obtains the fundamental enzyme peptidase from the paramecia that it ingests. Cathepsin II may similarly be taken over from the living food organism (Doyle & Patterson, unpublished).

(6) *Paramecium*

Literature on *Paramecium* up to 1931, comprising over 800 titles, has been brought together by Kalmus (1931); the culture methods up to that date are summarized and discussed. Hargitt & Fray (1917) were apparently the first to succeed in raising *Paramecium* on pure cultures of bacteria, which were isolated from hay infusions; they found that *P. caudatum* grew well on *Bacillus subtilis* but not on *Pseudomonas fluorescens*. Jollos (from Kalmus, 1931) grew *Paramecium caudatum* on *Chlorogonium* in Knop or Benecke solutions thus achieving pure 'zwiegleidrige' cultures. Leslie (1940 *a*, *b*) describes a medium suitable for the growth of *Paramecium multimicronucleata* on *Pseudomonas fluorescens*. Oehler (1920) obtained good mass cultures of *Paramecium* spp. on pure cultures of *Pseudomonas fluorescens*. Raffel (1930) has grown *Paramecium aurelia* on relatively pure cultures on *Bacillus candicans* and the alga *Stichococcus bacillaris*. Phelps (1934) attained for a limited time the pure culture of *Paramecium aurelia* on *Erythrobacillus prodigiosus*.

Johnson (1936) reported attempts to grow *Paramecium caudatum* in suspensions of various bacteria and an unknown yeast in a non-nutritive salt solution. Suspensions of *B. subtilis* alone supported good growth of *Paramecium*. Johnson thus confirms the findings of Hargitt & Fray (1917). However, in some cases there is definite evidence of marked nutritional variation between different strains of the same organism; thus Hargitt & Fray (1917) and Johnson (1936) found *Pseudomonas fluorescens* inadequate for *Paramecium* whereas Oehler (1924) and Leslie (1940) found it adequate, while Philips (1922) found *B. coli* inadequate whereas Chejfec (1929) found it adequate. Johnson (1941) tabulates the suitability of various bacteria as food for *Paramecium*. Hetherington (1934), working on *Colpidium colpoda*, concluded that the particular strain of food organism was as important as the species. Chejfec (1929) found that *Paramecium caudatum* requires a minimum of 24,000 *B. coli* per cubic mm. for maintenance of normal division rate in a single individual. 'Zweigliedrige' cultures of *Paramecium* have been described (Hetherington, 1932, 1933, 1934; Kidder & Stuart, 1939 *a*, *b*). Quantitative studies on the growth rate of *Paramecium* have been made by Phelps (1934), Leslie (1940 *a*, *b*), and Johnson (1936). Similar studies for *Glaucoma* were made by Phelps (1935, 1936). The relation of bacterial food to size of organism and fission rate has been described for *Glaucoma* (Harding, 1937 *a*, *b*) and for *Colpoda* (Kidder & Stuart, 1939 *a*, *b*).

The symbiotic zoochlorellae present in *Paramecium bursaria* afford an interesting special case. The nature of the symbiosis is reviewed by Loefer (1936 *a*, *b*) and Pringsheim (1928 *b*). Methods of culture are described by Pringsheim (1915, 1928 *a*, *b*) and Kalmus (1931). Pringsheim (1928 *b*) experimentally deprived *P. bursaria* of zoochlorellae and then reinfected the colourless form. The first bacteria-free culture was obtained by Loefer (1936 *a*), who found that the best growth was obtained on proteose peptone, next best on tryptone, and no growth with neopeptone or bactopeptone (1936 *b*, 1938 *b*). The importance of the concentration of salts was examined by Loefer (1936 *a*). Sodium acetate did not accelerate growth; of the carbohydrates tested, dextrose was best. Other hexoses were utilized but pentoses and inulin were toxic. The relation of *p*H to size and growth are given by Loefer (1938 *a*).

Between 1908 and 1930 a number of papers on the effects of various crude extracts of endocrine organs on the growth of ciliates appeared. In general an acceleration of growth occurs with all tissue extracts added. It is probable that the accelerations produced are due solely to the increased nutrient material which they convey (A. Lwoff, 1932). For the occurrence of acetylcholine and adrenalin in *Paramecium* see Koller (1938) and Schopfer (1939).

(7) *Glaucoma*

A detailed account of the methods of isolation and preparation of pure liquid cultures of *Glaucoma piriformis* is given by A. Lwoff (1932). It will grow on a number of so-called peptones prepared from hydrolysates of liver, spleen, muscle, silk, peanuts, brewers' yeast, etc. Hydrolysing agents include pepsin, trypsin, erepsin and mineral acids. Such peptones include proteoses, peptones, amino acids and other products. The proportions of the various constituents in a given preparation vary with the duration and type of hydrolysis. *Glaucoma* grows well on most of the above complex sources of nitrogen and carbon, but not on the purer silk hydrolysates, ereptones, or on mixtures of pure amino acids. Similar findings for *Colpidium* are reported by Elliot (1935 *b*).

Thiamin and riboflavin seem to be essential for growth of *C. campylum* in a de-ashed gelatin medium. Silk peptone and less highly purified gelatin supported growth without added growth factors (Hall, 1942). Hall (1939 *c*) found that pimelic acid caused acceleration of growth in *C. campylum*. This factor is required for growth on incomplete proteins such as gelatin, but with hydrolysed casein it causes no acceleration (Elliot, 1939; Hall, 1942).

A. Lwoff (1932) has set up nutritional balance-sheet experiments by analysis of such peptones before and during growth of the ciliates. He found that urea is not produced and any urea added to the medium is quantitatively recoverable. Ammonia is formed (cf. Doyle & Harding, 1937). Purine bases are produced after several days of growth, pre-

sumably from the autolysis of dead ciliates. Uric acid is not formed. Proteoses and peptones rapidly disappear from the medium and are to be considered the primary food source for the organism. A so-called X-fraction (that fraction of the nitrogen which is not precipitated by phosphotungstic acid, minus the amino, amide and ammonia nitrogen fractions), presumably peptides, at first increases in the medium and then decreases. Since the ciliates utilize such peptides it is possible that these substances arise by action of enzymes released from the ciliates, but whether or not the enzymes are excreted or arise by autolysis of dead *Glaucoma* is undetermined.

Certain carbohydrates are utilized by *Glaucoma* with accompanying fermentation. A. Lwoff (1932) and Loefer (1938*a*) found that *Colpidium* and *Glaucoma* metabolize dextrose, and the consumption of dextrose (Loefer) is greater than that found for trypanosomes by v. Brand (1935). Loefer & A. Lwoff agree that leucophytes do not utilize dextrose. Elliot (1935 *a*, *b*) found that only certain of the usable carbohydrates are fermented, as indicated by acid production. For the production of alkali by fatty acid ions from salts of fatty acids, see Barker, 1935, and Hutchens, 1941. Elliot found no indication of fermentation of dextrin, inulin, sucrose, lactose, salacin, melizitose, mannite, rhamnose, xylose or arabinose. The growth of *Colpidium striatum* is accelerated only by those carbohydrates which are fermented with acid production. Phelps (1936) found that the growth of *Glaucoma piriformis* is proportional to the concentration of nutrient within wide limits, and that it differs markedly from that of yeasts with respect to changes in concentration of waste products and food.

(8) *Amoebae*

The amoebae seem to resemble ciliates in nutritional requirements. Rice (1935, 1938 *a*, *b*) obtained excellent cultures of *Flabellula mira*, *Rugipes vivax*, a large and a small limax amoeba, on a diet of pure strains of various bacteria. A. Lwoff (1932) considered amoebae obligatory phagotrophs, but Reich (1933, 1935, 1936, 1938) cultured *Mayorella palestinensis* on a liquid medium of gelatin, peptone, dextrose, iron and other inorganic salts. Particles were removed with a Berkefeld filter. Reich concluded that *M. palestinensis* has food requirements analogous to *Glaucoma piriformis*.

Cailleau (1933 *a*, *b*, 1934) and A. Lwoff (1938*c*) have described the pure culture of *Acanthamoeba castellanii*. Cailleau (1933*b*) cultured this organism on rabbit serum and an extract of calf's liver; under these conditions added carbohydrates were not utilized. She (1934) also analysed the progressive changes in nitrogen fractions in media containing spleen, peanut and liver peptones. She found that during growth little ammonia was produced (thus differing from *Glaucoma* and *Leishmania*). The X-fraction increases. She concluded that *Acanthamoeba castellanii* utilizes the complex nitrogen precipitable by phosphotungstic acid. Vitamins of the B group were presumably present. In the Protozoa s.s. (Lw.) it has been reported (A. Lwoff, 1938*b*; Johnson, 1941) that *Glaucoma piriformis* and *Strigomonas* spp. require thiamin and are incapable of synthesizing it from its components, whereas *Acanthamoeba castellanii* requires the pyrimidine but may be able to synthesize the thiazol fraction, and that if both fractions are present the thiamin is unnecessary. Substituted derivatives of thiamin were found to be inactive (A. & M. Lwoff, 1937, 1938). Elliot (1939) found that riboflavin will not replace the thiamin which is required by *Colpidium striatum*. He found that prolonged autoclaving was required to remove all the thiamin from casein, and that gelatin plus thiamin was inadequate. Hall (1942) found that silk peptone Laroche contains thiamin so that the reported synthesis of thiamin by *Acanthamoeba* is questionable. She found also that riboflavin is required by *Colpidium*. Thus the vitamin requirements are directly related to the question of incomplete proteins as sources of food for the Protozoa.

(9) *Parasitic forms*

General culture methods and media for parasitic forms are to be found in Cailleau (1937 *c*, *d*), Hegner & Andrews (1930), and Wenyon (1926). Methods of culture of blood parasites and the role of haemoglobin are reviewed by Salle & Schmidt (1928) who further studied the changes produced by *Leishmania* in peptone media with and without carbohydrate. Marked protein degeneration, ammonia production and a protein-sparing action of carbohydrates were observed, *Leishmania* being similar in the above respects to *Glaucoma* (A. Lwoff, 1932 et seq.), *Acanthamoeba* (Cailleau, 1933*b*), and *Prototheca Zopfii* (Barker, 1935). Cailleau (1935, 1936 *a*, *b*, *c*, 1937 *a*, *b*, *c*, *d*, 1938 *a*, *b*, 1939 *a*, *b*, *c*, *d*) has investigated the nutritional requirements of *Trichomonas* spp. and *Eutrichomastix colubrorum*. These species were first grown on peptone bouillon plus an extract of tissue from the normal host species. Failure to obtain growth on bouillon plus blood sera from organisms other than the normal host was ascribed either to the presence of an agglutin or to the absence of a specific growth factor for *Trichomonas columbae* (1937*a*) (pure cholesterol was shown to be one, 1936*b*). Of 71 sterols tested, certain ones may replace cholesterol, others may not (1936*c*, 1937 *b*, *c*). The work up to 1937 is reviewed by A. Lwoff (1938*a*), who points out that in the suitability of a sterol the existence of a 5–6 double bond is not necessary, that OH at position 3 may be esterified without loss of activity, that if OH at 3 is removed or substituted by a ketone the activity is destroyed, that substitution at position 4 has no effect, and that the introduction of an alcohol or ketone group at position 6 or a ketone group at position 7 inactivates the molecule. Certain *Tricho-*

monas species require cholesterol or a related sterol (Cailleau, 1939*b*), and the need for ascorbic acid or a related substance has been shown for *T. foetus* and *T. columbae* (1938*a*, 1939*a*). Cultures of *T. gallinarum* were prepared bacteriologically pure by the use of sulphanilimide and quinanil. *Eutrichomastix colubrorum* requires cholesterol, a factor present in egg albumin, a factor from peptone and ascorbic acid (Cailleau, 1938*b*). Certain substances closely related to ascorbic acid may replace it, viz. *d*-iso-ascorbic acid, *d*-gluco-ascorbic acid, reductone, *d*-gluco-hepto-ascorbic acid, and reductinic acid (Cailleau, 1939*d*).

Nutritionally the trypanosomes fall into two main groups: (1) those capable of maintenance on peptone media, and (2) those requiring in addition blood or certain growth substances to be defined below which are found in blood. To the first group belong *Strigomonas* (*Leptomonas*) *oncopelti* (M. Lwoff, 1931*a*, 1933*a*), *S. culicidarum* var. *culicis* (M. Lwoff, 1935), *S. media* and *S. parva* (M. Lwoff, 1936). In the second group are *S. fasciculata* (A. Lwoff, 1933, M. Lwoff, 1931*b*, *c*, 1933*a*, *b*), *Leishmania tropica* (Salle & Schmidt, 1928), *Leptomonas ctenocephali* (M. Lwoff, 1932, 1933*a*), *L. pyrrochoris* (Zotta, 1923), *Strigomonas culicidarum* var. *anopheles* (M. Lwoff, 1935), *S. muscidarum* (M. Lwoff, 1936), *Trypanosoma lewisii* (Pannier, 1936), *T. rabinowitchi* (Nattan-Larrier & Dufour, 1936), *Schizotrypanum cruzi* (M. Lwoff, 1938*a*), and *Leishmania* spp. (M. Lwoff, 1939). All forms in this second group failed to grow in peptone alone, but the addition of varying amounts of blood gave good growth through several transfers. Haematin (Merck) can replace whole blood (M. Lwoff, 1932). A smaller quantity of haematin than of blood is required for growth. M. Lwoff (1931*b*) suggested that haematin behaves as a peroxidase. Subsequent experiments (M. Lwoff, 1931*c*, 1933*a*), showed that Wolff's peroxidase when stabilized by addition of gum arabic and certain vegetable peroxidases could replace haematin.

A. Lwoff (1933) showed that for *Strigomonas fasciculata* the addition of blood to a peptone medium increases the respiration and the division rate. The increase in respiration is proportional to the quantity of blood added. Addition of blood to a glucose medium causes a very slight increase. Haematin, protohaematin and protoporphyrin also increase respiration while haematoporphyrin, mesoporphyrin, rhodine, pheophorbide, pyroporphyrin, aetioporphyrin, deuteroporphyrin, deutohaemin, mesohaemin, pheophorbide-*a* haemin, pheophorbide-*b* haemin and pyrohaemin are without effect. A. Lwoff (1936) ascribes the non-multiplication without blood to a deficiency in the respiratory system owing to lack of ability of the organisms to synthesize protoporphyrin. The medium (containing iron) must supply peptone plus protoporphyrin or protohaemin or the prosthetic group of haemoglobin. M. Lwoff (1933*b*) found that protohaemin could not be replaced by another haemin. Zotta (1923), found that for *Leptomonas* calf's liver could replace blood.

The fact that the trypanosomes are nutritionally like *Glaucoma* and other free-living Protozoa s.s., except that most of the trypanosomes are unable to complete their respiratory enzyme system without the addition of suitable porphyrin, is an instance of biochemical parasitism (M. Lwoff, 1933*a*). There is, however, no correlation between the ability of the trypanosome to synthesize porphyrin and the blood-sucking habit of its host. For example, *Leptomonas pyrrochoris* and *L. oncopelti* are both parasites of vegetarian Hemiptera, yet the former is incapable of synthesizing haemin and requires the addition of blood to the peptone medium, while the latter does not (M. Lwoff, 1936). Similarly, this inability to synthesize haemin has been used as one of the factors in separating two varieties of *Strigomonas culicidarum* (M. Lwoff, 1935). *S. culicidarum* var. *culicis* is found in the stomach of omnivorous larvae of *Culex pipiens* and does not require the addition of blood when cultured on a peptone medium. *Strigomonas culicidarum* var. *anophelis*, which is found in the stomach of adult blood-sucking or vegetarian *Anopheles quadrimaculata*, is incapable of synthesizing haemin and requires the addition of blood to a peptone medium. Subsequently M. Lwoff (1938*a*) substituted serum alone and washed red corpuscles alone for whole blood and found that these gave negative results. However, the substitution of serum and washed red blood cells gave as good cultures as whole blood (M. Lwoff, 1938*a*). Similar results were obtained for *Leishmania* spp. Further experiments in the substitution of various compounds for blood have shown that the combinations of serum + haemin, serum + *l*-ascorbic acid, haemin + *l*-ascorbic acid, added to the peptone medium in the culture of *Schizotrypanum cruzi* (M. Lwoff, 1938*a*) and *Leishmania* spp. (M. Lwoff, 1939) gave negative results, while the combination of serum + haemin + ascorbic acid gave positive results. In comparing the growth requirements of *Strigomonas* spp. and *Leptomonas* spp. with those of blood bacteria M. Lwoff (1933*a*) stated that the factor 'V' which was necessary for the bacteria had not been shown to be essential for these flagellates. In later work (M. Lwoff, 1937, 1938*b*) she found that thiamin is a necessary factor for growth, and further that *Strigomonas* spp. could not substitute pyrimidine and thiazol for thiamin. Studies of a similar nature on bacteria have been made by A. Lwoff & Pirosky (1937), A. Lwoff & Querido (1938, 1939), Monoyer (1937), Querido, A. Lwoff & Lataste (1939).

Thus in the development of parasitism there is in the case of *Strigomonas* a loss of the ability to synthesize an integral part of an enzyme system, the haem system, just as the Protozoa s.s. have lost the ability to synthesize thiamin, and *Didinium* has lost the ability to synthesize peptidase (see below).

IV. BIOCHEMISTRY

Contributions to protozoan nutrition are to be found in the fields of general metabolism, intermediary metabolism, respiration and enzyme chemistry. The literature on general metabolism of the Protozoa has been thoroughly reviewed by v. Brand (1935), and only certain subsequent references are included here.

Quantitative data on ammonia production by ciliates have been reported by Specht (1934) for *Spirostomum* and by Doyle & Harding (1937) for *Glaucoma*. The intermediary metabolic processes upon which the nutritional requirements of these organisms are based have as yet barely been outlined. The majority of the numerous studies on respiration cannot be related to nutritional states of the organism or to precisely determined nutrient substrates. In general Protozoa exhibit two types of respiratory mechanism. In some the respiration is strongly inhibited by cyanide and carbon monoxide, e.g. the trypanosomes *Strigomonas* and *Leptomonas* (Cailleau, 1937 *c*, *d*). In others, e.g. *Trigomonas*, *Paramecium*, *Colpidium*, *Glaucoma*, the respiration is cyanide-insensitive (see Cailleau, 1937 *d*). Concerning other organisms which are similarly cyanide-insensitive, and for the significance of this in relation to metabolism, see Oppenheimer & Stern (1939, p. 260). Some of the respiration studies treat of intermediary metabolism in certain of its aspects. Barker's (1935) results on *Prototheca* have already been discussed. Salle & Schmidt (1928), M. Lwoff (1934) and Hutchens (1941) present results more directly bearing on nutritional aspects. Hutchens (1940, 1941) found cytochrome *C* and cytochrome oxidase present in *Chilomonas paramecium*, and in consideration of the respiratory quotient and growth rate has related the utilization of iron and thiamin to this oxidative system. Hutchens, Jandorf & Hastings (1941) have reported synthesis of diphosphopyridine nucleotide by *Chilomonas*.

The larger part of our information on the intermediary metabolism of the Protozoa has resulted from the application of Pringsheim's concept of *Azetatorganismen* and Lwoff's *oxytrophie*. Acetate is utilized in the formation of reserve carbohydrate as well as in more immediate combustion. Whereas Pringsheim and Mainx (1926) thought that sugar in the medium was an intermediary in starch formation, A. Lwoff (1938 *a*) maintains that sugar cannot replace acetate in the formation of reserve carbohydrates in leucophytes. Salle & Schmidt (1928) reported a protein-sparing action of *Leishmania* in the presence of carbohydrates. Loefer's (1938 *a*) work on the fermentation of carbohydrates by *Chilomonas*, *Colpidium* and *Glaucoma* has been reviewed above. The claim of Mast & Pace (1933) that *Chilomonas* oxidizes ammonium salts to nitrite and nitrate has been refuted (Waksman, 1940), so that no protozoan is known to be chemoautotrophic in the sense of maintaining itself on energy from inorganic ions and carbon dioxide with or without light. That *Chilomonas* is capable of utilizing carbon dioxide in the absence of light seems to be definitely substantiated (Burrows, 1938). The role of carbon dioxide in the carbohydrate cycle of mammalian tissues and of green plants in darkness has been recently extended (Franck & Gaffron, 1941; Solomon, Vennesland, Klemperer, Buchanan & Hastings 1941; Van Niel, 1941) and might be expected in Protozoa under similar conditions. Incorporation of carbon dioxide in the carbohydrate cycle requires the presence of appropriate energy sources. Demonstration that an organism is capable of a given fermentation or of utilization of a particular substance is to be distinguished from determination of the maintenance requirements. Experiments of short duration demonstrating the existence of synthetic and oxidative powers should be carried out in relation to the nutritional state of the organisms.

A complete description of the nutritional characteristics of an organism requires a knowledge not only of the nature of the medium capable of supplying all constituents necessary to continued maintenance but also requires a knowledge of the various hydrolytic and oxidative enzyme systems involved. The ability of organisms to synthesize certain components of vitamin molecules may be lost without changing the type of metabolic pathway concerned. Certain of the vitamins are constituents of fundamental enzyme systems. When the ability to synthesize such a constituent is lost, the organism must find the constituent in the medium or utilize an alternative metabolic pathway. In the case of the ciliate *Didinium* it appears that a fundamental enzyme, peptidase, cannot be synthesized by the organism and that the enzyme is taken over intact from its living food, namely *Paramecium* (Doyle & Patterson, 1942). The identification of extra- and intracellular hydrolytic enzymes of the organisms indicates to some extent the nature of nutritional reactions. In the Protozoa it is necessary to modify the usual categories since digestive enzymes which are extracellular in higher forms may be active either outside the protozoan (in the medium) or inside it (in the vacuoles) and intracellular enzymes are only those actually in the cytoplasm. The nature and existence of carbohydrate food reserves (glycogen, paramylum, starch) in an organism give some indication of intermediary processes and predicate the existence of certain types of enzymes.

Relatively few of the enzyme studies on Protozoa have been carried out with the use of biochemical procedures. Many of the papers are observations on morphological changes in food vacuoles which indicate the presence of enzymes. The formation and fate of the food vacuole and its contents, the changes in acidity and the behaviour of associated neutral red granules have been described by Nirenstein (1905, 1910), Mast & Doyle (1935 *b*), Mast (1938 *a*), Hopkins (1938), Volkonsky (1933 *a*) and Claff, Dewey & Kidder (1941). During the second or alkaline

stage of the food vacuole some of the food material dissolves. Frequently the food organism contains substances identifiable microscopically. Starch and paramylum disappear after becoming etched. Hydrolysis is indicated by the changes in staining reaction with iodine. Cosmovici (1931*a*) performed an interesting experiment on a ciliate by placing it in a medium of starch paste of such consistency that the food vacuoles were not pinched off but finally formed a tube. Starch at the stomal end of the tube stained blue with iodine whereas that at the anal end was red or colourless. Oil or fat droplets have also been observed to disappear from vacuoles, and although few tests have been made on changes occurring in the oil the appearance of new fat deposits in the cytoplasm after an oil meal indicates assimilation of the material. Dawson & Belkin (1928, 1929) fed *Amoeba dubia* and *A. proteus* on a series of known oils and found that some were digested and others not, and that certain differences existed between the two species.

The protein portions of food organisms have been similarly studied and the fate of known coagulated proteins has been followed. These substances generally dissolve. From the acidity of the food vacuole as judged by indicators different authors have variously concluded that the Protozoa in question contained tryptic or peptic proteinases. The next step towards identification of the proteinases was the preparation of extracts of crushed cells. Hartog & Dixon (1893) with *Pelomyxa* found that their extract would liquefy gelatin in weakly acid media and concluded that pepsin was present. Mouton (1902) with *Amoeba zymophila* determined the *p*H optimum for liquefaction of gelatin by cell extract and found it to be *p*H 7·6. He concluded that a tryptic enzyme was present. Sawano (1938) investigated the proteolytic system of *Paramecium* in some detail. Using washed organisms recently fed on egg yolk he prepared both glycerine extracts and dried powders. Sawano found no indication of pepsin or trypsin but found that catheptic proteinase (*p*H optimum 4·6), catheptic carboxy and amino polypeptidases, and dipeptidase were present. Lawrie (1935, 1937) used washed cytolysed *Glaucoma* and found a proteinase of the papain group (*p*H optimum 6·0) which was active on casein but not on egg albumin. He also found a peroxidase and a dehydrogenase. Doyle & Harding (1937) found evidence of a rapid evolution of ammonia by recently fed *Glaucoma*, indicating proteoclastic activity in the food vacuoles. Holter & Kopac (1937) demonstrated that the peptidase in *Amoeba* is localized in the hyaline cytoplasm. Holter & Doyle (1938 *a*, *b*) studied the properties and distribution of amylase and peptidase in *Amoeba proteus*, *Paramecium caudatum* and *Frontonia* sp. and found that the distribution of amylase is different from that of peptidase. Unfortunately physiological variation renders localization methods difficult. Topological evidence has been presented (Horning, 1937 and others) indicating a relation between hydrolytic enzymes and mitochondria. Such a localization has not as yet been confirmed by enzymatic histochemical methods for proteolytic enzymes but there are indications that amylase in amoeba may be so localized. Further evidence is required and may be forthcoming from the use of synthetic substrates.

With organisms in biochemically defined pure culture it is possible to some extent to determine the nature of the enzyme from the configurations of the substrate molecules which can be utilized. Thus α- and β-glucosidases have been detected in bacteria. The presence of adaptive enzymes (Knight, 1936) in bacteria raises problems of immediate pertinence to the analysis of protozoan nutrition data.

In the culture media of Protozoa hydrolytic enzymes are frequently present. Many workers on intestinal Protozoa maintain that enzymes are excreted by the Protozoa but in relatively few instances have these organisms been grown in the absence of bacteria (Ratcliffe in Hegner & Andrews, 1930). On the other hand gelatine in the culture medium is liquefied by *Mayorella* (Reich, 1935) and *Glaucoma* (A. Lwoff, 1932). In cases of culture media analysed before and after the growth of Protozoa (*Glaucoma*, A. Lwoff, 1932 and Loefer, 1938*a*; *Acanthamoeba*, Cailleau, 1933 *a*, *b*, 1934) a degradation of protein occurs far in excess of the nitrogen built into the Protozoa; some of this degradation may be due to enzymes freed from autolysing organisms, some may be due to enzymes excreted with remnants of food or in some other fashion.

V. OLIGODYNAMIC SUBSTANCES AND ALLELOCATALYTIC EFFECTS

In addition to suitable carbon and nitrogen sources, the common minerals, etc., the Protozoa require for their maintenance unknown numbers of trace substances. When quantitative methods are applied to the estimation of growth the importance of these trace substances becomes greatly magnified. One of the most confusing aspects of the literature is that dealing with allelocatalysis. That an observed growth rate is dependent on small details of the culture method is not surprising, nor has it been seriously questioned. But it has been vigorously contested that Protozoa produce substances in the culture fluid which accelerate their own growth or that the production of such substances can be determined by varying the size of the original inoculum. It has, however, been established for a number of forms since the early work of Robertson, Peterson *et al.* that the size of the original inoculum affects the rate of growth (see Pratt, 1940; Ludwig & Boost, 1939; Mast & Pace, 1938*b*, *c*; Kidder, 1941; Reich, 1938; and Dusi, 1940). That increased growth resulting from large inocula is due to production of allelocatalysts as maintained by Robertson and by

Reich (1938) *et al.*, has been vigorously denied by Chejfec (1929), Darby (1930*b*), Jahn (1934*b*), Johnson (1933), and Phelps (1935, 1936). These authors variously ascribe the observed growth acceleration to differences in the amount, kind or availability of the food under the various conditions of the experiments. The more recent studies have endeavoured to relate observed growth promotion and inhibition to various features of the culture procedure.

It must be borne in mind that in the growth of a protozoan culture the condition of the medium is known only at the start of the experiment. It is apparent that if fresh medium contains certain growth inhibitors, such as toxic ions, supra-optimal concentrations of foodstuffs, etc., the inhibiting action of these substances may be reduced by growth or by the presence of larger inocula. Growth of the organisms would thus result in production of a biologically conditioned medium giving growth acceleration. If a fresh medium is lacking in certain essential but oligodynamic substances these may be provided by autolysis of a fraction of the inoculum, thus accelerating a limited growth. It is obvious that growth may produce a depletion of some essential of the medium thus giving higher growth rates with smaller inocula.

In addition to the above considerations there remains the observed effects simply of the ageing of sterile organic media. In such chemically complicated solutions it may be expected that new molecular species will result from the ageing of the solution. Ageing may also be expected to remove the more reactive species of chemical compounds, not all of which are deleterious. Ageing in the presence of growing organisms will obviously have significant effects on chemical composition of the medium in addition to changes due to actual absorption of nutrients. Enzymes will be released into the medium, possibly by excretion and certainly by autolysis of dying forms, and in complicated organic media the effects of many of these may be profound. Excretory end-products, in addition to direct effects upon growing organisms, will alter the reaction mixture, and may also participate in the extracellular enzymatic reactions.

With the above effects ruled out the evidence to date appears to me to be definitely opposed to the existence of allelocatalysts in the strict sense of the term. Hall & Loefer (1940) found that simple ageing of the casein peptone media causes acceleration of growth rate. Unlike the accelerants found by Mast & Pace (1938*c*) and by Kidder (1941), that found by Hall & Loefer is thermostable. The production of an auto-inhibitor for growth of *Chlorella* in a well-controlled culture fluid is reported by Pratt (1940). In *Glaucoma* Hall (1941*a*), and Hall & Shottenfeld (1941), found that vitamin deficiency inhibits growth. Obviously large numbers of substances are required for growth which will act as stimulants when added to media suboptimal in concentration. To the generalizations and special cases noted above should be added work on pantothenic acid (Hall & Elliot, 1935), bios and vitamins (Elliot, 1937*a*, 1938, 1939), pimelic acid (Hall, 1939*c*, 1942), iron (Dusi, 1940) and agar factor (Leonian & Lilly, 1940).

VI. EVOLUTION OF NUTRITIONAL TYPES

The intermediary position of the protozoan forms here considered, between the plants and animals, and at a stage of structural complexity, and perhaps physiological limitation, above the bacteria, presents a unique opportunity for comparative studies. Knight (1936) has dealt with comparative requirements of bacteria and certain Protozoa. The fungi show many requirements paralleling those of Protozoa (see Leonian & Lilly, 1940; Robbins & Kavanaugh, 1938; Schopfer, 1939; and Volkonsky, 1933*b*). Similar problems are met in the culture of excised plant tissues (White, 1939). Factors known to affect plants have been tested on Protozoa. Elliot (1937*b*, 1938) compared the effects of certain auxins on the holophytic *Euglena gracilis*, the saprozoic *Khawkinea halli* and the holozoic *Colpidium striatum*. The plant hormones markedly inhibited growth of *Colpidium* and less so that of *Khawkinea*. On the other hand, *Euglena* showed marked growth acceleration with γ-3,*n*-indole butyric; β-3,indole propionic and 3,indole acetic acids. Coinciding with variations in nutritional requirements are variations in cytoplasmic structure (Lwoff, 1938*a*). Further evidence of the relation of plastid structure to nutritional requirements is to be found in Oltmanns (1905, 1922), Volkonsky (1930 *a*, *b*, *c*), Pringsheim (1921, 1937*d*), A. Lwoff (1932, 1935*a*, 1938*a*), A. Lwoff & Dusi (1934). For cytological criteria of the plastid see Guilliermond, Mangenot & Plantefol (1933).

Euglena Mesnili may be grown in the light as a photometatroph (A. Lwoff & Dusi, 1935*a*). Under these conditions it possesses about 100 chloroplasts. After six months in darkness the number of plastids is reduced to 15 or 20, and after fifteen months most organisms have two plastids and some rare individuals have none and no longer contain starch. In some such manner the Protozoa s.s. Lw. may have evolved from chlorophytes or from leucophytes. The following generalizations have been made: 'All colourless phytomastigophora are capable of heteroautotrophic (Hl.) nutrition' (Hall, 1939*b*). Within the class of heterotrophs (Hl., Pr.) A. Lwoff (1938*a*) has stated that all leucophytes are oxytrophs, all chlorophytes experimentally deprived of the chlorophyll function are oxytrophs (A. Lwoff, 1932, considered these haplotrophs), no chlorophytes or leucophytes are haplotrophs, no Protozoa s.s. Lw. are oxytrophs.

VII. SUMMARY

The principal feature of recent studies on the nutrition of the Protozoa has been the development of culture media suitable for the maintenance of several score of species in 'pure culture'. As investigators approached precise definition of the constitution of the culture media the significance of 'trace elements', accessory food factors and the like became more apparent. The types of culture apparatus, the methods of sterilization of organism and medium, the procedures of estimating growth of populations, and the exact composition of reagents have all been found to be of greater importance than originally realized. Within the phylum there exist chlorophyll-bearing, plant-like forms and numerous intermediary types up to and including those requiring living particulate food, thus providing the full range of nutritional types. The older systems of physiological classification have been extensively revised, but none of those proposed is adequate. In some instances the relations between variations in cytoplasmic structure (plastids) and nutritional requirements have been demonstrated.

Many species of chlorophyll-bearing and colourless flagellates have been studied. Intensive work on *Polytoma*, *Chilomonas* and *Euglena* has revealed notable species and strain variations with respect to factors such as fatty acid metabolism, nitrogen requirements, the effects of light on nutrient requirements and on response to plant hormones and vitamin constituents. The effect of systematic modification of chemical structure on the utilization of vitamin components has been studied for some species. A few dozen of the normally phagotrophic (holozoic) forms have been grown in the absence of other living forms (pure liquid culture), while certain forms have been grown on single species of food organisms (pure mixed culture) or on dead food organisms. With the exception of certain amoebae the salient work on pure culture has been carried out with the smaller ciliates, particularly *Glaucoma*. The media employed (protein hydrolysates, tissue autolysates, etc.) are chemically and physically highly complex. Such media contain labile constituents susceptible to important changes when subjected to minor procedural variations. Analyses of the media before and after the growth of organisms have given some indications of the nature of the nitrogenous fractions metabolized. The accessory growth-factor requirements of these forms are complex. The very specialized nutrients required by parasitic forms have demonstrated interesting evolutionary relationships. An increasing number of biochemical experiments is being reported on the existence of various enzyme systems in the Protozoa. Our knowledge, however, of the metabolism of representative Protozoa remains fragmentary.

VIII. REFERENCES

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BIOLOGICAL RACES IN PARASITIC PROTOZOA

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I. INTRODUCTION

The system of classification, accepted at present both by botanists and by zoologists, is based on the degree of *morphological* divergence between living organisms, which are assigned to species or lower systematic categories according to the magnitude of these structural differences. It is well known, however, that there exist, among animals and plants, groups which differ from each other in *biological* characters only but appear to be identical in structure. Unless their biological peculiarities are known, such groups, being indistinguishable by the usual morphological criteria, remain undetected and cannot be differentiated from each other. Thus, a systematist comparing morphologically identical specimens from diverse populations, and being unaware of any difference in their bionomics, would attribute them all to one and the same species.

Up to the present there is no agreement regarding the exact status of the groups in question, which are designated loosely as biological, physiological, ecological or host (also hostal) races or species, none of which has a recognized taxonomic status. Biological races, as we shall refer to them, occur among both free-living and parasitic organisms, examples of the latter being much more numerous, owing to their economic (agricultural or medical) importance, on account of which they have been studied more thoroughly. The features characterizing biological races vary considerably, but, generally speaking, they are based on ecological preferences. Thus, some free-living organisms (e.g. 'ecotypes' in plants) may differ in occupation of a restricted habitat and form distinct populations adapted to diverse edaphic and climatic conditions. Others (e.g. races of *Drosophila funebris*) are viable at different temperature ranges. Parasites (external and internal) may differ in their food preferences, in more or less rigid host-specificity (or host-restriction), or localization within the same host (organ or tissue specificity), as well as in their effect upon the host. Furthermore, parasitic bacteria and Protozoa may be distinguished serologically. These are but a few examples of the great variety of characters which serve to differentiate biological races.

Here it may be mentioned that some authors describe as biological races groups of organisms which, in addition to bionomic divergence, differ also in minor structural characters (Thorpe, 1930; Brierley, 1931; Huxley, 1940). But since groups which are separable by differences in structure—however slight these may be—can be placed in one of the existing minor systematic units, their inclusion among biological races is unjustified, for these are by definition restricted to populations indistinguishable morphologically. For this reason allied groups should not be defined as biological races without a thorough examination, to determine that they do not actually differ in some minute structural characters and might not belong to some other intraspecific category. The best example of such a case is provided by two races of the plant louse, *Chermes abietis*, which differ markedly in their life cycles. Having failed to discover any morphological difference between these groups, Cholodkovsky (1907) regarded them as 'biological species'. Since these were considered to be equivalent to conventional species, he split the original species into two, *C. abietis* and *C. viridis*. However, Philiptschenko (1916), who studied these insects by statistical methods, demonstrated the existence of certain structural differences and accordingly referred these races to two morphological subspecies, *C. abietis abietis* and *C. abietis viridis*.

It is possible that the differences between biological races are actually correlated with cytological features which are not revealed by the technique at our disposal. Thus, two biological races of *Drosophila pseudoobscura*, which are indistinguishable by any external morphological trait, were found to differ in their chromosomal constitution (cf. Dobzhansky, 1941). In the course of the evolution of such races cryptic structural changes may be gradually succeeded by visible characters. Once this stage is reached morphological criteria can be used for classification on the orthodox lines. But as long as structural divergence remains undetectable and only biological differential characters are available, it is convenient to separate such groups—in which morphological differentiation is, as it were, *in statu nascendi*—from the conventional morphological groups under the name 'biological races', for want of a better independent taxonomic unit.

Biological races can, therefore, be defined as such subdivisions of a morphological species as are distinguishable by differences in biological characters only.

II. GENERAL CONSIDERATIONS

In the animal kingdom biological races are well known among ectoparasitic and phytophagous insects, and among parasitic helminths (Thorpe, 1930, 1931, 1940; Chandler, 1923; Goodey, 1931). They

are quite common in some Protophyta, especially among bacteria (White, 1931; Topley & Wilson 1936) and the lower fungi (Ramsbottom, 1926; Brierley, 1931). Although such races also occur among parasitic Protozoa, not many biologists are aware of their existence, mainly owing to the fact that the best-known examples are found among the pathogenic parasites of man and domestic animals, which belong to a rather highly specialized branch of medical zoology. The true nature of biological races in parasitic Protozoa is, moreover, obscured because, in view of their medical and veterinary importance, the clinical and pathological aspects of infections with these microorganisms have unduly influenced their classification. As the result of this, groups indistinguishable morphologically are in many cases described as independent species. In fact most of the parasitic groups in question have been treated as independent 'good' species for such a long time, especially by medical and veterinary workers, that any attempt to interpret them as biological races, with the consequent reduction of the number of existing species, will come as a revelation to some, and may meet with opposition from those whose views on systematics have an anthropocentric bias.

As far as I am aware, the question regarding biological races in parasitic Protozoa has never been the subject of a special review. I therefore propose to give an account of this phenomenon mainly as observed in Protozoa pathogenic to man and domestic animals, as these forms have been more thoroughly investigated. The present review is not, however, entirely analytical, but to a large extent constructive. In view of the indeterminate status of these races, the opportunity is taken to define their position in relation to the morphological groups, with the ultimate object of incorporating them in the hierarchy of taxonomic units, with an independent rank.

The description which follows, though representative, is by no means exhaustive, while the references throughout this article are not always to original publications, but rather to general works or to the most recent ones in which the points under consideration are adequately dealt with.

Since examples of biological races in parasitic Protozoa are unevenly distributed among various systematic groups, it will be more convenient to consider them according to the abundance and variety of types of biological divergence than in the order of their position in the system. As will be seen, the great majority of biological races are found among the blood-inhabiting Protozoa.

III. HAEMOFLAGELLATES

Biological differentiation is well represented in certain haemoflagellates of the family Trypanosomidae (Mastigophora), the trypanosomes and leishmanias.

The best-known examples of the former are three species, *Trypanosoma gambiense*, *T. rhodesiense* and *T. brucei*. The first and second are human parasites causing two types of sleeping sickness in tropical Africa, while the last-named causes the disease nagana in hoofed animals. These trypanosomes are morphologically indistinguishable from each other. The only difference between the three species of the *brucei* group—as it is sometimes described—is biological, and consists in the relationship of these trypanosomes to their hosts. Though all three are infective to various mammals and may occur naturally in antelopes, only *T. gambiense* and *T. rhodesiense* are capable of infecting man, whereas *T. brucei* is restricted to other mammals. All attempts to infect human volunteers with *T. brucei* have invariably failed, the few reports of accidental infection of man with this trypanosome being entirely unfounded. *T. gambiense* and *T. rhodesiense*, though both parasites of man, differ from each other in various physiological features, mainly in virulence. Whereas *T. gambiense* produces in man the classical sleeping sickness, which is typically a chronic disease with well-marked nervous symptoms, *T. rhodesiense* causes an acute form of disease, usually without involving the central nervous system, but with toxic symptoms. The behaviour of the two trypanosomes in experimental infections of rodents is likewise different: while *T. rhodesiense* is very virulent, *T. gambiense* does not readily infect them. On the other hand, *T. brucei*, though differing from both human trypanosomes in its inability to infect man, behaves in laboratory animals in every way like *T. rhodesiense*. It is, therefore, more closely related to this species than to *T. gambiense*.

From these facts it is evident that the three trypanosomes of the *brucei* group, though assigned to three independent species, are actually biological races of one and the same species, *T. brucei*. Although biological races are at present without a recognized taxonomic status, we have complied with the Law of Priority (Art. 28: Rules of Zoological Nomenclature) in the choice of names for the species to which these and other biological races to be described below have been referred. In following this course biological races have been treated as an intraspecific unit, in anticipation of their recognition as a formal systematic category with a distinct name. In the present case the choice is also in harmony with the most acceptable hypothesis regarding the mutual affinities of these trypanosomes. From the available data it would appear that the human trypanosomes have originated from *T. brucei*, probably from some antelope strain. *T. gambiense* had presumably established itself in man a long time ago, whereas *T. rhodesiense* has only recently evolved from *T. brucei* (Duke, 1921, 1936*a*, 1936*b*; Reichenow, 1939; Wenyon, 1926).

Somewhat similar relations are observed among trypanosomes of the *evansi* group. Members of this group comprise parasites of various mammals, the

most important being horses, camels and cattle, to which the trypanosomes may be pathogenic in varying degrees. There is no universal agreement as to the number of species in this group. However, the diseases caused by them belong to two distinct types. One, dourine, is a venereal disease which occurs in equines exclusively and is common in some parts of Asia, North Africa and southern Europe. The causative organism, *T. equiperdum*, is found chiefly in connective tissue exudates, but not in the blood of the host. Its transmission is effected without the intervention of insect vectors, but from mammal to mammal by contact during the sexual act. The second disease is generally known as surra, though various local names are also applied to it (e.g. Mbori, Tahaga, El Debab, Su-Auru, etc.). It is widely distributed both in the Old and in the New World. The trypanosomes, which produce a blood infection in the host (equines, bovines and camels) and are transmitted mechanically by horse-flies (Tabanidae), have been attributed to a number of independent species, mainly on the grounds of serological differences between them. The chief of these are as follows: *T. evansi*, *T. soudanense*, *T. marocanum*, *T. aegyptum*, *T. annamense*, *T. ninae kohl-yakimov*, *T. su-auru*, *T. hippicum*, *T. venezuelense*, *T. equinum*. With the exception of the last-named, all these species are morphologically identical and, since the type of disease produced by them is also similar, many authorities rightly recognize only *T. evansi* as a valid species. *T. equinum*, which differs in structure from the other members of the *evansi* group, need not be further considered.

The remaining species of the group, now reduced to two—*T. equiperdum* and *T. evansi*—have retained their independent status, in spite of the fact that they too are morphologically indistinguishable. As mentioned already, the type of disease caused by these two parasites, their habitat in the host and method of transmission, differ considerably, but since these biological features are the only criteria by which they can be distinguished, it is evident that *T. equiperdum* and *T. evansi* represent merely biological races of one and the same species, for which the older name, *T. evansi*, should be retained. The relations between the surra race and dourine race of *T. evansi* are thus of the same order as those between the two human races of *T. brucei* (*gambiense* and *rhodesiense*), but the biological divergence between the two trypanosomes of the ungulates, manifested in the diseases they produce, is greater than that between the two human trypanosomes.

There is a further subdivision within the surra group itself, for it comprises geographical strains which differ from each other in their effect upon various mammals. In some localities (Indo-China, Java) horses suffer more than cattle, the disease in the former being acute and in the latter mild or chronic (Laveran & Mesnil, 1912). In other countries (Anglo-Egyptian Sudan, Somaliland), on the contrary, horses are immune to infection, the effect upon cattle is slight, but camels are highly susceptible hosts (Hoare & Bennett, 1937). Conditions in India appear to be variable, for in some parts of the country the effect of surra upon equines and bovines is similar to that in Indo-China and Java (Edwards, 1926), while in the Punjab the course of the infection in camels, horses and cattle shows the same differences as in the Sudan and Somaliland (Cross, 1921). On the other hand, in North Africa horses and camels are equally affected (Barotte, 1925). The biological differences between various local strains of the surra race of *T. evansi* are thus manifested in the degree of their pathogenicity to different mammalian hosts. These characteristics, though constant, are not so pronounced as the features distinguishing this race from the dourine race (*T. equiperdum*), on the one hand, and those which serve to differentiate members of the *brucei* group, on the other.

The next group to be considered are haemoflagellates belonging to the genus *Leishmania*. The best-known representatives of this genus are parasitic in man and in dog (as well as in some other mammals). These parasites cause two types of disease: one, in which the infection is generalized, is known as visceral leishmaniasis or kala azar; the other, with a localized infection, is known as cutaneous leishmaniasis or oriental sore. The two diseases, which are sharply differentiated clinically and pathologically, are widely distributed in warm countries all over the world. They are attributed to the following species: *L. donovani* and *L. chagasi*, causing the visceral human disease in the Old and New Worlds respectively; and *L. tropica* and *L. brasiliensis*, responsible for the cutaneous human disease in the corresponding parts of the globe. Some authors recognize still another species, *L. infantum*, for the Mediterranean type of kala azar which affects almost exclusively young children and dogs. This species is differentiated from the Indian *L. donovani*, because the latter affects mainly adult persons and does not occur in local dogs. Distinct names have also been given to the parasites of canine leishmaniasis: *L. canis* for the one causing the visceral form, and *L. tropica* var. *canina* for that causing cutaneous lesions (Laveran, 1917; Wenyon, 1926; Chagas, Cunha, Castro, Ferreira & Romaña, 1937).

Here again the criteria for classification are purely medical, for the flagellates of the species named above are not only morphologically identical but all attempts to differentiate them by their behaviour in culture and by serological methods have also failed (Chodukin, Sofiev & Kevorkov, 1935). In fact, the only clue to the 'species' of parasite is provided by the clinical picture of the case from which it was obtained. However, some of these 'species' have not even this criterion to recommend them. Thus the disease caused by *L. chagasi* is identical with that caused by *L. donovani*, while *L. brasiliensis* and *L. tropica* both provoke local lesions. As regards the

canine parasites, there can be no doubt that they are identical with the ones producing human leishmaniasis. This identity has been established experimentally, and it is possible that dogs serve as reservoirs from which human beings may acquire their infection. The elimination of these redundant species leaves us with two, *L. donovani* and *L. tropica*. From the facts considered above it is evident that the human and canine parasites described under these names actually represent two biological races of the same species, for which the earlier name, *L. donovani*, should be reserved. One of these races produces a generalized visceral infection, while the other gives rise to localized cutaneous or mucosal lesions. The dissimilarity between these two diseases is greater than that between the two types of sleeping sickness in man, but less than that between dourine and surra in equines.

IV. HAEMOSPORIDIA

Further instances of biological races are provided by some of the blood-inhabiting Sporozoa of the order Haemosporidia, especially by the malaria parasites and piroplasms.

The anthropoid apes, chimpanzee and gorilla, harbour three types of malaria parasites which are morphologically identical with the three species commonly found in man: *Plasmodium falciparum*, causing malignant tertian malaria; *P. vivax*, causing benign tertian malaria; and *P. malariae*, causing quartan malaria (Reichenow, 1939). In order to determine the mutual relationships between the human and simian parasites a number of cross-infection experiments have been carried out (Rodhain, 1939, 1940). In the case of the malignant tertian parasites attempts to infect man from chimpanzee have so far failed. As regards the benign tertian form, chimpanzees could be infected with the human parasite, but man was found to be refractory to infection with the simian strain. On the other hand, man proved to be susceptible to infection with the quartan parasite of the chimpanzee, though the human strain of this parasite did not produce infection in the apes. These experiments have also demonstrated that *P. vivax*, when introduced from man into chimpanzee, produced in the latter an inapparent or cryptic infection which could only be revealed by subsequent infection of another human being from the ape. However, the quartan parasite of chimpanzee provoked in man febrile symptoms similar to those in human malaria. Some authors (cf. Brumpt, 1939) maintain that, because of the high degree of host-restriction,* the human and simian malaria parasites should be regarded as distinct species. The parasites of the apes were accordingly given the following names: *P. reichenowi* for the malignant tertian form; *P. schwetzi* for the benign tertian form; and *P. rodhaini* for the quartan one. However, in view of the absence of any morphological difference between them, it is obvious that the human and the simian parasites, especially those of the chimpanzee, are limited to three species, each comprising two biological races, one adapted to man, the other to apes. The specific names to be borne by the malaria parasites of these primates, in accordance with the Law of Priority, should be those applied to the human forms (*P. falciparum*, *P. vivax* and *P. malariae*).

In addition to the races of malaria parasites differing in host-restriction, there occur in man geographical strains of *Plasmodium*, which differ from each other in biological features but not structurally (Boyd, 1940; Coatney & Young, 1941; James & Ciuca, 1939; James, Nicol & Shute, 1932). Thus, strains of *P. falciparum* from Italy, India and West Africa differ from each other in the clinical symptoms they produce (virulence), in their susceptibility to drugs and immunological reactions. A Dutch strain of *P. vivax* was found to be less virulent than one from Madagascar, the two strains also differing in the length of the incubation period and infectivity to mosquitoes. Comparable differences have been established between strains of human malaria parasites isolated in various parts of America. Furthermore, it has been demonstrated that even strains from the same locality, but derived from different patients, may be immunologically distinct. Among the avian malaria parasites a new variety, *P. relictum* var. *matutinum*, was recently created for a strain isolated from robins (Huff, 1937). This strain agrees morphologically with the original species, but differs from it in various biological features, such as periodicity of asexual cycle, virulence to canaries and infectivity for mosquitoes. This variety therefore represents a typical biological race.

Among piroplasms the most interesting instances of biological differences are found in parasites of the genus *Theileria* causing east coast fever in bovines (Carpano, 1937; Du Toit, 1931; Sergent, Donatien, Parrot, Lestoquard & Plantureux, 1927; Wenyon, 1926). The classical type of this disease, as observed in South Africa, is caused by *T. parva*, which is highly pathogenic, producing in cattle an acute disease with a high mortality rate. On recovery the bovines acquire complete immunity. The vector of *T. parva* is a tick of the genus *Rhipicephalus*. A similar type of disease, prevalent in North Africa and in Asia, is attributed to another species, *Theileria dispar* (= *T. annulata*) which is transmitted by ticks of a different genus (*Hyalomma*). Finally, a third parasite, *Theileria mutans*, is non-pathogenic, producing in cattle a chronic benign infection which confers only partial immunity (premunition), and is transmitted by *Rhipicephalus*. This mild form of theileriasis occurs in Africa, Asia and southern Europe. As is known, in certain stages of their development piroplasms of the

* We follow Culbertson (1941) in adopting 'restriction' in preference to 'specificity', in view of the different connotations of the latter term in biology.

genus *Theileria* live within the erythrocytes of their host, in which they appear as minute rounded or rod-shaped forms, the proportion and number of which vary according to species. The parasites are numerous in infections with *T. parva* and *T. dispar*, but scanty in *T. mutans*. Moreover, in *T. parva* the predominant forms are rod-shaped (82%), while in *T. dispar* the commonest forms are the rounded ones (92%). It is generally admitted that the three species of bovine *Theileria* are morphologically indistinguishable, the differences just noted being purely relative. Nevertheless, these quantitative characters and the biological differences already described (course of disease, distinct vectors, type of immunity) have been used as criteria for the differentiation of these piroplasms as independent species. There can be no doubt, however, that they should be regarded as biological races of the older species *T. parva*, manifesting various degrees of pathogenicity to bovines and adapted to transmission by different intermediate hosts. Similar differences have been noted in two piroplasms of sheep and goats, one of which, *T. ovis*, is non-pathogenic, while the other, *T. hirci*, is pathogenic (Thomson & Hall, 1933). Since they are identical in structure they represent biological races of *T. hirci*.

Biological races also occur among piroplasms of the genus *Babesia* (Reichenow, 1935). They differ from each other in their restriction to particular intermediate hosts. Thus, European strains of the canine piroplasm, *B. canis*, are transmitted by ticks of the genus *Dermacentor*, whereas an Asiatic strain was found to be transmissible only by ticks of the genus *Rhipicephalus*. The Asiatic strain, which was named *Babesia major*, is also less pathogenic than the European one. Similar relations were found in *B. bigemina*, the piroplasm responsible for red-water or Texas fever in cattle. Some strains of this species are transmitted by ticks of the genus *Boophilus*, others by those of *Rhipicephalus*. On account of this, it was suggested that these strains represented independent species, though no names were given to them.

V. INTESTINAL PROTOZOA

The intestinal parasites provide only a few records of authentic biological races, i.e. of groups in which the existence of biological divergence, coupled with morphological identity, has been established beyond doubt. The most striking instances are Protozoa parasitic in man and frog (Dobell, 1918). The dysentery amoeba of man, *Entamoeba histolytica*, and the intestinal amoeba of frogs, *E. ranarum*, are morphologically identical, both in the active stage and in cysts. There is likewise no structural difference between the human and batrachian intestinal flagellates, *Chilomastix mesnili* and *C. caulleryi* respectively. All attempts to infect tadpoles with the human parasites have invariably failed, thus proving that they were refractory to infection. The human strains therefore represent races adapted to man, whereas the corresponding batrachian ones are probably races adapted to frogs. These cases are remarkable because the hosts—one a warm-blooded mammal, the other a cold-blooded amphibian—are so widely separated zoologically and physiologically. If the Law of Priority is strictly applied, the human and batrachian amoebae and flagellates in question would have to be regarded as biological races of two species, *Entamoeba ranarum* and *Chilomastix caulleryi* respectively.

VI. STRAINS OF UNCERTAIN POSITION

In all the cases considered so far the available data leave no doubt that we are dealing with biological races as defined in the introductory section. In some instances, however, morphologically indistinguishable groups of Protozoa have been attributed to independent species on grounds of biological differences between them, but without sufficient evidence for their recognition as biological races. Thus, there is no structural difference between the intestinal amoebae of mice (*Entamoeba muris*), guinea-pigs (*E. cobayae*) and man (*E. coli*), and similarly between the murine and human intestinal flagellates, *Chilomastix bettencourti* and *C. mesnili* (Wenyon, 1926). The various species of amoebae and of flagellates named above, therefore, actually represent a single species of *Entamoeba* and *Chilomastix* respectively, comparable to *Entamoeba debliecki*, an amoeba comprising strains parasitic in pig and in goat (Hoare, 1940). In the absence of experimental evidence of host-restriction it is impossible to determine whether these species and strains represent biological races or heteroxenous parasites, such as the ciliate, *Balantidium coli*, which is common to man, monkeys and pigs (Wenyon, 1926), or the amoebae (*Entamoeba histolytica*, *E. coli* and *Endolimax nana*) and species of *Trichomonas*, which inhabit both man and macaque monkeys (Dobell, 1931, 1933, 1934, 1936). The strains within each of these species are interchangeable between the various hosts in which they are capable of living.

The position of some other Protozoa is still more debatable. Some authors (cf. Brumpt, 1936) believe that man may be infected with two amoebae forming quadrinucleate cysts. One, for which the name *Entamoeba histolytica* is reserved, is the causative organism of amoebic dysentery, while the other, separated under the name *E. dispar*, is said to be non-pathogenic. The existence of a special non-pathogenic race—morphologically indistinguishable from the pathogenic form—is, however, disputed by most observers. There is also considerable controversy regarding the mutual affinities of the human and simian flagellates, *Trichomonas hominis* parasitic in the intestine, and *T. vaginalis* inhabiting the vagina. While some authors (e.g. Chandler, 1923; Dobell, 1934) believe that these trichomonads represent physiological races of one and the same species, others (e.g. Stabler, Feo & Rakoff, 1941) maintain

that they are specifically distinct. Adherents of each of these conflicting views base their arguments on experimental data.

VII. DISCUSSION

In the foregoing account numerous instances are given of biological races occurring in various groups of parasitic Protozoa. It has been shown that the features which serve to distinguish intraspecific races vary considerably. Some of these races manifest a more or less rigid host-restriction or specificity (either to the final or to the intermediate host), others give rise to different diseases in the same host. In the latter category are also races which have a distinct localization in definite organs or tissues, as well as races differing merely in the degree of virulence or pathogenicity. Finally, in certain races some of these differential characters may be combined. In general, these biological races may be said to differ in various aspects of their host-parasite relations. This relationship is the fundamental feature of all forms of parasitism, the special branch of ecology in which one living organism (the host) constitutes the environment or habitat of another (the parasite). The body of the host thus comprises the sum total of the conditions which determine the existence of the parasite. As a rule, the factors of the external environment (temperature, humidity, light, etc.) have no direct effect upon the parasite (except temporarily, in the exogenous phases), but influence it through the medium of the host. Furthermore, this association, unlike that between free-living organisms and the outside world, involves reciprocal reactions between parasite and host, bringing into play various specific agents. While parasites belonging to morphologically distinct groups may also differ from each other in physiological properties, in biological races the peculiarities of the host-parasite relations are their only differential characters. A closer examination of these relations will, therefore, help to throw more light on the nature of the races in question.

In the case of races differing in host-restriction (or specificity), each race is physiologically adjusted to conditions in its natural host, but not to those in others. The natural host provides the parasite with optimum physical and chemical conditions, such as temperature, food, etc. Moreover, the parasite is equipped to withstand the defence mechanism of the host (phagocytes, antibodies). In a non-susceptible animal (or plant) various factors may prevent a parasite from setting up an infection. Thus, the character of the digestive juices may not be suitable for hatching the cysts of intestinal Protozoa (Culbertson, 1941; Hegner, 1927), or the serum may possess natural parasiticidal properties (cf. Topley & Wilson, 1936) which affords protection against infection with blood Protozoa. It is known, for instance, that the oocysts of coccidia, when ingested by animals other than the natural hosts, pass unchanged through the alimentary tract. The resistance of man to infection with *Trypanosoma brucei* is probably due to the fact that normal human serum kills this trypanosome, whereas it has no effect upon *T. gambiense*. The other race, *T. rhodesiense*, appears to occupy an intermediate position: in the human body it resists the action of serum, but may lose this property after passages through other mammals (Yorke, Adams & Murgatroyd, 1930).

The adaptation of different races to their hosts appears to vary considerably in degree. Such variation is observed in races which have a wide choice of hosts but show a preference for a limited number (e.g. geographical races of *T. evansi*; human and simian races of *Plasmodium*), and in races which, though inhabiting the same host, produce in it distinct diseases (*Leishmania*) or differ in pathogenicity (*Theileria*).

Amongst the factors which influence the occurrence of parasites in their hosts nutrition should also be mentioned. It is known that qualitative and quantitative variations in the host's diet may have a profound effect on the course of parasitic infections. Thus, the presence of certain vitamins in the food may inhibit the development of rat coccidia (Becker, 1941), while a carnivorous diet leads to a marked diminution in the number of trichomonad flagellates in rats (Hegner, 1927). It is conceivable that difference of diet may also be responsible for the relative host-restriction observed in *Balantidium coli*. This intestinal parasite occurs chiefly in domestic pigs, to which it is harmless, and occasionally in man, to whom it may be pathogenic. Since this ciliate feeds mainly on a starch diet it thrives best in the intestine of the pig, where it finds abundant and suitable food. Judging from the fact that balantidial infection is rare in man, it would appear that the conditions in the human bowel are not very favourable to the existence of the ciliate, probably owing to the scarcity of starch. It is thought that insufficiency of suitable food in the lumen of the bowel induces the ciliate to attack its walls, with the result that the pathological symptoms of human balantidiosis are produced (Dogiel & Gnesdiloff, 1935).

Our knowledge regarding the mechanism of adaptation of parasites to the environmental conditions in the host in general is very limited, but certain aspects of the reciprocal reactions between host and parasite have been more fully elucidated, especially in bacteriology. Thus the study of the serological reactions of the host has served as a basis for the analysis of the antigenic composition of diverse microorganisms, resulting in a deeper knowledge of their intimate constitution and *modus operandi* (cf. Topley & Wilson, 1936; Landsteiner, 1936).

It is known that certain species of pathogenic bacteria comprise distinct types (or populations) which may differ in host-restriction (e.g. *Brucella* in goats, cattle or pigs; *Salmonella*), in the type of disease produced in the same host (e.g. *Bacterium dysenteriae*), or in virulence (e.g. the pneumococci

= *Streptococcus pneumoniae*). These 'types'—being morphologically indistinguishable, but differing in their relations to the host—are thus equivalent to biological races in parasitic Protozoa. It has been established that types belonging to the same species of bacteria differ from each other in antigenic composition. Antigenic constitution is, therefore, correlated with the various pathological and immunological manifestations which these bacteria produce in the host and which represent their differential characters. In most cases, moreover, it has been demonstrated that all the types within a given species have certain antigens in common ('group' antigens), while others are peculiar to each of them individually ('specific' or 'type' antigens). Each species as a whole can, therefore, be characterized by the common or 'group' antigens, while the differential physiological characters of the types or biological races belonging to the same species are determined by their 'specific' antigens. Furthermore, it has been shown that antigenic differences depend mainly upon the chemical structure of the constituent proteins, which may be combined with polysaccharides or lipoids (haptens). From these facts it may be concluded that in bacteria the distinction between types or biological races resolves itself into differences in the chemical constitution of their antigenic components.

Although antigenic analysis has only recently been applied to Protozoa, the available data give reason to assume that in these microorganisms the differences between biological races and strains, at any rate among haemoprotozoa, are determined by the same factors as in bacteria. Variation in the antigenic composition accounts for the rapid adaptation of trypanosomes to antibodies produced by the host in the course of infection ('relapse strains': Ritz, 1916), as well as for the more lasting differences between intraspecific strains of trypanosomes and malaria parasites, as reflected in the strain-specificity of the immune response of the host (Taliaferro, 1941; Hornby, 1941; Sinton, 1937; James & Ciuca, 1939; Manwell & Goldstein, 1939). A closer insight into antigens of trypanosomes has been given by recent studies (Kligler & Olitzki, 1936; Kligler, Olitzki & Kligler, 1940; Broom & Brown, 1940), indicating that the serological individuality of these flagellates is due to the predominance of lipoid components in the antigen complex, and that strains of the same species contain both group and type (or racial) antigens. Both on the strength of these facts and on analogy with bacteria, it is permissible to assume that the ultimate difference between biological races of parasitic Protozoa is likewise chemical.

The question now arises whether these biological races represent stable groups with constant characters, or variable groups. Our knowledge of some races (e.g. trypanosomes of the *brucei* and *evansi* groups, and of *Leishmania* spp.) extends back to about half a century. In the course of this period—during which these parasites have been practically under continuous observation—their biological features have remained unaltered. Since in the history of a unicellular animal this period amounts almost to eternity, we are justified in concluding that these races have stood the test of time and are hereditarily fixed. This fixity does not, however, exclude the evolution of new strains or races from existing ones, probably by mutation as in the higher organisms. That physiologically separable races differ in their genetic make-up and are hereditarily stable seems to be well established among other protists (e.g. in bacteria: Topley & Wilson, 1936; in rust fungi: Gowen, 1933).

Thus, as regards hereditary stability of characters, biological races do not differ from morphologically distinguishable systematic units with constant characters. However, certain biological groups (e.g. relapse strains in trypanosomes), which are environmentally induced and unstable, are comparable to enduring (or persistent) modifications (*Dauermodifikationen*), and taxonomically equivalent to morphae. The permanence of true biological races in Protozoa must be specially emphasized because of the current impression that biological divergence among the protists is generally transient (cf. Robson, 1928; Huxley, 1942).

Throughout the foregoing account biological races have been treated as intraspecific units, in anticipation of the formal recognition of their taxonomic status. The desirability of assigning these races to an independent systematic position, with a distinct name, is based not only on the peculiarity of the differential criteria (biological, instead of morphological) but also on the fact that their diagnostic characters are constant and hereditarily fixed. Lack of space prevents further discussion of this problem, but it is proposed to deal with it more fully in a separate publication.

VIII. SUMMARY

Biological races are defined as such subdivisions of a morphological species as are distinguishable by differences in biological characters only. Though biological races are well represented in diverse groups of parasitic Protozoa, their true nature is in most cases concealed under separate specific names.

In parasitic Protozoa biological races differ from each other in various aspects of their host-parasite relations. These may be manifested in host-restriction (or host-specificity), as in trypanosomes of the *brucei* group, containing races confined to man and to other mammals; in intestinal amoebae and flagellates comprising human and batrachian races; and in human and simian malaria parasites (*Plasmodium*). Likewise, races parasitic in the same vertebrate host may be restricted to distinct vectors, as in piroplasms (*Babesia*) of cattle and dogs.

There are races which cause different diseases in the same host, usually with a distinct localization in its tissues, e.g. *Leishmania* in man and dogs, and trypanosomes of the *evansi* group, causing dourine and surra. Others again vary in the degree of virulence to the host, e.g. piroplasms of the genera *Theileria* and *Babesia*. A variation in pathogenicity to one or more hosts charac-

terizes some geographical races of the same parasite, e.g. local strains of human *Plasmodium*, and of *Trypanosoma evansi*. Finally, in certain races various differential characters may be combined.

Variations in the host-parasite relationship, as manifested by different races of Protozoa, are determined by the degree of their adaptation to the physical and chemical conditions in the host. These conditions include, among others, body temperature, quality of the digestive juices, nutrition (diet), and properties of the serum (parasiticidal action). The mutual adaptation of parasite and host involves reciprocal reactions, among which the serological reactions of the host are especially interesting, for they throw light on the intimate constitution of parasitic microorganisms. There is reason to believe that biological races of some parasitic Protozoa—like the intraspecific 'types' of bacteria—differ from each other in antigenic composition, while retaining common antigens characteristic of the species as a whole. Antigenic constitution is, therefore, correlated with the biological peculiarities of these races (at least in blood parasites), and since antigens consist of qualitatively different chemical substances, the distinction between biological races resolves itself into differences in the chemical structure of the antigenic components of the organisms in question.

Biological races in parasitic Protozoa appear to be stable and hereditarily fixed. The biological criteria for their differentiation and the constancy of their diagnostic characters are advanced as arguments in support of an independent taxonomic status for biological races.

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TIMBER DECAY*

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I. HISTORICAL INTRODUCTION

Knowledge of the chemical and physical changes which occur during the decay of timber, and of the fungi which bring about these changes, has advanced rapidly during the past 15 years or so, the period covered by this survey.

Between the classical researches of R. Hartig, who discovered the true nature of decay in wood and described the role of fungi in causing tree diseases, and the intensive research work of the last few decades, there is a wide and rather sterile gap, during which individual workers in various countries made isolated investigations on specific problems. The monumental work of Falck (1907–27) on *Merulius lacrymans* and other fungi which cause dry rot in buildings is a landmark which leaves little more to be done on these particular organisms. The practical application of Falck's results was made available to the general public in this country by Groom (1927), who had made a special study of the German botanical literature and was well qualified to interpret it.

The study of timber decay arose as an offshoot from the study of tree diseases involving the trunk, and while there is, of course, no sharp dividing line between timber and forest pathology, in recent years some workers have tended to specialize and concern themselves rather more with the study of decay in felled timber. The establishment in various parts of the world of Forest Products Laboratories, i.e. institutes in which the properties and defects of timber as a material are studied from every angle, has led to a great development of research on timber decay, and it is largely as a result of their work that our knowledge of this subject has progressed so rapidly in recent years.

II. SYLVICULTURAL CONSIDERATIONS

It is beyond the scope of this article to consider in any detail the sylvicultural conditions which lead to the development of decay in standing trees, but it is satisfactory to note that the importance of maintaining healthy growing conditions by correct sylvicultural practice, thereby ensuring healthy trees with good quality timber, is being better appreciated by foresters.

The importance of felling a stand of trees as soon as the amount of rotting exceeds the rate of increment of the trees has been brought out in studies by Boyce (1932), who has shown that in certain stands of Douglas fir the gross increment per acre keeps well ahead of the rot increment until an age of somewhere between 300 and 350 years is reached, when the latter equals it, and from then on exceeds it. It is not only in comparatively undeveloped countries, where virgin stands still exist which are becoming over-mature, that this is of importance, for there are indications that butt rot of conifers in this country becomes significant under certain circumstances only when a certain age is reached. Generalizing, it may be said that crown rot of a tree becomes serious only as the trees become over-mature, while butt rot is liable to attack comparatively young trees if the latter are growing under unfavourable conditions. Thus it is mainly with butt rot that the forester in this country is concerned. Day (1934) has quoted many examples of how an unsuitable environment affects the incidence of disease.

III. DIAGNOSIS OF CAUSE OF WOOD ROTS BY PURE CULTURAL METHODS

The first problem which confronts the investigator of decay in timber is that of identifying the causal organism, especially when the rot is in an early stage. Hubert (1924) was one of the first to make a comparative study of the effects of various fungi on the microscopic appearance of the cell walls, and he published a useful table based on such characters as size of bore holes and presence or absence of clamp connexions on the hyphae in the wood. The examination of fungal mycelium in wood has been facilitated by the discovery of new techniques for staining and mounting of microscopic preparations. One of the simplest and most effective of these is the safranin and picro-aniline blue method described by Cartwright (1929). It is, however, only in a few species that the microscopic appearance of the mycelium and its effect on the wood are sufficiently characteristic to enable the organism to be identified, and in order to do so it is usually necessary to prepare cultures of the fungus. The majority of wood-rotting fungi do not fructify readily on ordinary media and it is necessary to have available for comparison either an extensive collection of named cultures, or accurate, well-

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illustrated descriptions of the cultures. The detailed and accurate descriptions of cultures of a number of important wood-rotting fungi in Canada published by Fritz (1923) provided an excellent basis for future work, and her paper is an important landmark in the study of wood-rotting fungi. In similar work along these lines Cartwright & Findlay (1930) have shown that it is possible to draw up diagnostic keys based on the cultural characteristics of the fungi. They also pointed out (1934) how certain physiological characteristics such as the optimum and maximum temperatures for growth and the rate of growth are useful aids in distinguishing between species in culture; for instance, the 'wild' species of *Merulius—M. sylvester* —is able to grow at 30° C. while the dry-rot fungus, *M. lacrymans*, does not grow at temperatures above 25° C. Humphrey & Siggers (1933) listed a large number of wood fungi according to their temperature relations. The chemical reactions induced by bacteria have long been recognized as useful criteria for their diagnosis, and this method has been used to some extent for characterizing fungi in culture; for instance, Bavendamm (1928) showed that the brown colour produced by a fungus when grown on a medium containing tannic or gallic acid indicates the presence of an oxidase, and that this enzyme is produced only by fungi which bring about white rots in wood (see p. 149). It may be noted here that the addition of a few drops of guaiacum solution to a liquid culture, giving a blue colour in the presence of oxidase, is an even more delicate test for distinguishing between cultures of fungi which cause brown rots and those which cause white rots.

The smell of certain cultures is highly characteristic, and Badcock (1939) has listed a number of species which can be recognized by the odours which they produce in culture. W. A. Campbell (1938) has pointed out how the different species of *Fomes* in culture produce distinct characters which make their identification fairly certain, and has drawn attention to the value of cultural studies not only for the diagnosis of the cause of decay but also in ordinary taxonomic work for distinguishing between species closely related in external form and for identifying abnormal or abortive forms of fungi. It is in ill-defined groups such as *Poria*, which possesses comparatively few morphological characteristics, that cultural studies are particularly useful, and Baxter, in a long series of papers, has made full use of cultures for characterizing the species of *Poria* which he has described. Refshauge & Proctor (1935) published a series of keys to certain wood-destroying fungi occurring in Australia, based on their appearance when grown on various agar media. While it may be possible to develop a reliable key so long as it is restricted to a certain group, the number of features available and subject to accurate description is limited, so that it is generally preferable to restrict any key of this kind to a group of fungi occurring on one host or on a series of related hosts. This method has been adopted by Cartwright and Findlay, who have given in keys and tabular form the diagnostic characters in culture of the fungi which cause rot in oak (1936), in coniferous timbers (1938) and in other hardwoods (1942).

In the absence of a collection of standard cultures prepared from authentically named sporophores, the only means of recognizing the identity of an unknown species in culture is by its producing sporophores sufficiently typical to enable it to be recognized. A number of investigators have studied the conditions influencing their formation, and generalizing it may be said that the following conditions must be fulfilled in varying degrees for each species: (1) a rich, well-aerated medium; (2) a relatively high moisture content around the growing mycelium; (3) an atmosphere of moderately high humidity, but not one saturated with moisture, around the developing sporophores; (4) exposure to light of an intensity and duration which is related to the natural habitat of the fungus.

Etter (1929) described a bread medium on which she found these fungi fructified readily, and recently Badcock (1941) has shown that sawdust with the addition of a small percentage of maize meal and bone flour provides an excellent medium for the cultivation of most wood-rotting fungi, and one on which sporophores are readily produced if other conditions are favourable.

IV. GENERAL DESCRIPTIVE WORK

It is impossible within the scope of this article to refer to all the general descriptive work which has been carried out on the wood rots in the past few years; a great deal of this relates to heart rots of trees and is primarily of sylvicultural interest. Much of this scattered information relating to decays of timbers used in this country has been brought together and critically reviewed in a series of papers by Cartwright & Findlay (1936, 1938, 1942). The heart rots of conifers in Norway have recently been fully described in a monograph by Jørstad & Juul (1939).

In America pioneer work in describing and classifying the commoner rots of timber was done by von Schrenk & Spaulding (1909), and since then the subject has been further extended by Humphrey, Snell, Hubert, Boyce, Zellner, Schmitz, Richards, Colley, and many others. Hubert (1931) and Boyce (1938) have both written textbooks in which this accumulating knowledge has been sifted and critically summarized. In the nineteen-thirties an important series of papers on the heart rots in the forests of the Eastern U.S.A. appeared from the Bureau of Plant Industry in Washington under the names of Hartley, Davidson, Campbell and Blaisdell, and others. Their studies have been associated with forest improvement work by the Civilian Conservation Corps, and one of the interesting conclusions they have drawn from this work is that there is a very much greater risk of decay developing in trees grown from sprout shoots than

there is in trees raised from seedlings. These workers have described a number of hitherto unrecognized rots and have extensively employed cultural methods for their recognition and characterization. Space does not permit the listing of many other important contributions to the study of forest and timber fungi which have been published by the Forest Experiment Stations and University Schools of Forestry in the U.S.A.

In Germany, Liese at Eberswalde, has investigated the butt rots of conifers and the so-called 'frost heart' in beech, and has written extensively on many aspects of timber and forest pathology. In the last few years a number of lengthy papers which contain few original ideas have been published by a number of workers in Germany.

In a study of the fungi which rot railway sleepers in Poland, Walek Czernecka (1933) gave detailed and excellent comparative descriptions of the causal fungi and their microscopic characters. The fungi which grow on timber in damp mines have interested a number of mycologists on account of the curious abnormal forms which the sporophores may assume under conditions of high humidity in the absence of light. Pilat (1927) has studied the flora of the Přibram mines in Czechoslovakia and described many 'forms' of the species which occur there.

Of the value of recent Russian work on this subject it is difficult for the authors of this review to judge, since these papers have been read only in summary or abstract form. Much, however, has been published, and Vanine (1930) has written a text-book on wood rot describing the principal decays of timber in Russia. A considerable amount of systematic work on the taxonomy of the Polyporaceae has been carried out by Russian workers, which should help to clear up the unsatisfactory nomenclature of this group, which includes many species important as destroyers of wood.

Hemmi in Japan has done a good deal of descriptive work and (1932) published a longish paper on the fungi which attack conifers in Japan; a number of other Japanese workers have contributed to this subject.

Summing up, one may say that on the purely descriptive side great progress has been made during the last 10–15 years, and that the publication of handbooks and papers collating the results of different workers and listing descriptions in a comparative way with diagnostic keys has enhanced the value of a great deal of the descriptive work by individual workers.

V. PHYSIOLOGY OF WOOD-ROTTING FUNGI

(1) *Effects of temperature relations.* It has already been indicated how the different cardinal temperatures for the growth of wood-rotting fungi may provide useful criteria for the recognition of certain species in culture. Lindgren (1933) has suggested that the optimum temperature for the growth of a fungus may be different on agar and in wood, but his data do not appear to be statistically significant. It has been found that the nature of the medium has, in general, little or no influence on the temperature relations of fungi.

In connexion with the sterilization of timber infected with decay, the thermal death point of various fungi has been determined. It has been found that the time required at various temperatures to kill most fungi varies considerably according to the physical condition of the medium in which the fungus is growing; generally speaking, the drier the medium the higher the temperatures or the longer the time required. Most of the tests have been carried out with agar cultures or with blocks of wood containing a high moisture content. Using infected blocks one inch cube of moist wood, Montgomery (1936) found that *Lentinus lepideus*, the most resistant of the fungi tested, was killed by an exposure for 60 min. to a temperature of 65° C. There seems to be a sudden increase between 60° and 65° C. in the effect of temperature on these fungi, so that much shorter times are required to sterilize wood at 65° than at 60° C.

Chidester (1937, 1939) carried out a series of tests in order to determine the length of time necessary to kill various fungi in wood, which were known to be resistant to heat. *Lenzites trabea*, *L. sepiaria* and *Lentinus lepideus* appeared to be most resistant of the fungi tested. She concluded that it is not practical to sterilize wood by using 'internal' temperatures lower than 150° F. (about 65° C.). Provided that the atmosphere around the wood was more or less saturated, so that little loss of water could take place, the following times and temperatures were found to sterilize the wood, even if it was infected with the most resistant of the fungi: 150° F. 75 min.; 170° F. 30 min.; 180° F. 20 min.; 200° F. 10 min.; 212° F. 5 min.

(2) *Nutritional requirements.* The nutritional requirements of wood-rotting fungi have been studied from two angles: (1) by pure culture studies; (2) by chemical analysis of the effect of the fungi on wood; this latter aspect is dealt with on p. 151.

It has been found that wood-rotting fungi can derive their energy from a wide range of polysaccharides. Glucose, as might be expected, is probably the sugar most favourable for their growth. Starch is readily attacked, as are many of the hemicellulose compounds. Some difficulty has been experienced in obtaining satisfactory growth on chemically prepared cellulose, although it is evident that this substance in the wood is readily attacked (see p. 152). Pentosans are also capable of acting as a source of energy.

The favourable effect on the growth of these fungi in wood of an increased content of nitrogen, particularly in an organic form in the wood, was demonstrated by Findlay (1934), and has since been confirmed by Schmitz & Kaufert (1936). The utilization

of various nitrogen compounds was studied by La Fuze (1937), who found that some species utilize amino and ammonia nitrogen better than amide or nitrate nitrogen. The important discovery by Kögl & Fries (1937) that many Basidiomycetes are able to utilize inorganic forms of nitrogen only in the presence of aneurin (vitamin B_1), makes it necessary to reconsider in the light of this discovery much of the earlier work on the nitrogen metabolism of these fungi. Fries (1938) later extended the scope of these investigations and demonstrated the relation between various doses of aneurin and biotin and the response to this vitamin given by different fungi. He also showed that the presence of relatively small numbers of certain bacteria greatly stimulates the growth of some fungi in synthetic liquid media.

The specificity of many wood-rotting fungi vis-à-vis their hosts has long been a subject of enquiry. Some species, such as *Polystictus versicolor*, are able to grow on almost any kind of timber; others, particularly those which cause heart rots, are highly specific and grow on one species or genus of plants. Lutz (1925 et seq.), in a long series of papers, has shown that this specificity can be directly associated with the ability of the fungus to grow in the presence of certain toxic substances such as tannin, and that if samples of a timber such as oak are thoroughly leached with water they become liable to attack by many other fungi (see p. 154). Although this conclusion appears generally to be true and it may be shown by experiment (Cartwright, 1937) that certain fungi such as *Fistulina hepatica*, which normally occurs only on oak and chestnut, can grow in a medium containing a comparatively high concentration of tannin, which inhibits the growth of most other fungi, yet many puzzling cases of specificity remain to be explained. Why, for instance, is *Polyporus betulinus* in nature almost completely confined to birch, when it can be made to grow on a variety of woods in the laboratory? As far as is known, birch does not contain a substance which is toxic to other fungi; it may be that birch wood contains substances especially favourable for the growth of *P. betulinus* or it may be that the physical conditions, such as the moisture and air relationships, in the birch tree are peculiarly suited to this particular fungus; or again, it may be that it does in fact grow on other trees, but since the conditions there are unfavourable for the formation of its sporophores, it escapes detection. Further work on this interesting problem is required.

(3) *Hydrogen-ion concentration and growth.* It has long been known that most wood-rotting fungi render distinctly acid any medium in which they are growing and that they will make good growth only in a medium which is on the acid side of neutrality. These fungi vary considerably in the amounts of acid they produce; in general those which bring about a brown rot are capable of bringing the *p*H down to a lower figure than those which cause a white rot. The *p*H of an extract of wood decayed by *Merulius lacrymans* or *Coniophora cerebella* may be 3·0 or even lower, while that of wood severely rotted by *Polystictus versicolor* is usually about 4·5. In liquid media the acidity produced by brown rot fungi may persist for several months, but with white rot fungi, which possess an oxidase system, this initial increase in acidity soon tends to disappear, and the medium reverts to the initial *p*H, presumably as a result of the oxidation of the acids after depletion of the sugars; this reversal of *p*H change does not appear to take place when the fungi are growing in wood. Oxalic acid is probably the commonest organic acid produced by wood-rotting fungi, but Birkinshaw, Findlay & Webb (1940) have shown that *Coniophora cerebella*, when growing in Scots pine sapwood, can produce considerable amounts of citric acid.

The spores of many Hymenomycetes require acid conditions in order to germinate, and some of the wood-rotting forms (e.g. *Merulius lacrymans*) require a strongly acid solution for the germination of their spores. It has been suggested by Curtin (1927 et seq.) that the production of acids by wood fungi may play an important part in rendering soluble and actively toxic, antiseptic substances, such as zinc meta arsenite, which have been deposited in an insoluble 'fixed' condition in the wood.

(4) *Growth in relation to moisture content of substratum.* It is extremely difficult to carry out carefully controlled experiments to determine the optimum moisture content for the growth of wood-rotting fungi, because these fungi by their action on the wood produce water of metabolism by breaking down the carbohydrates, and they thus raise the moisture content of the medium on which they are growing. Control of the atmospheric humidity around the test specimens is only effective when moisture contents below the fibre saturation point (about 27 % of the dry weight in most woods) are required, and the optimum moisture content for the growth of most of these fungi lies considerably above this point.

For the general run of fungi moisture contents of between 35 and 50 % (of the dry weight) are the most favourable for growth, though a few species, such as *Coniophora cerebella* and *Paxillus panuoides*, make better growth when the moisture content is higher still. The optimum moisture content for the growth of any particular fungus varies from one species of timber to another, depending on the density of the wood; in a dense timber a comparatively low moisture content may almost fill up the lumina of the cells and thus retard the growth of the fungus by reducing its oxygen supply, while in a very light, porous timber a very much higher moisture content still leaves sufficient space for the air necessary for the growth of the fungus. Thus there is a balance between the moisture and air requirements which varies from one specimen of timber to another, and it is not possible to lay down any exact optimum moisture content for any species of fungus except in relation to timbers of similar density and structure. For this reason experi-

ments which have been carried out using sawdust instead of solid wood give results which are quite meaningless when applied to solid wood. On account of the much greater porosity of sawdust, the optimum moisture content for fungal growth in it is usually of the order of 200% of the dry weight.

It is possible to determine the minimum moisture for growth with somewhat greater accuracy than the optimum, but here again there is a complication, for the minimum moisture content at which spores may germinate and set up decay is somewhat higher than the minimum at which the fungal mycelium can infect a timber by growing on to it from an adjoining focus of infection. Practical tests in experimental floors have shown (Findlay, 1937) that there is no risk of *Merulius lacrymans* spreading to timber if its moisture content is below 20%, and that there is very little chance of decay becoming initially established unless the moisture content is somewhat higher than this, e.g. probably round about the fibre saturation point.

Wood-rotting fungi are essentially aerobic organisms, and if wood is saturated with water there is no risk of its becoming decayed, although these fungi may remain alive for a considerable time under waterlogged conditions. The species of fungi vary considerably inter se, in their resistance to anaerobic conditions. *M. lacrymans*, for instance, is intolerant of waterlogged conditions and is never found growing in very wet wood; *Armillaria mellea*, on the other hand, can be observed on mine timbers which are saturated with water running over them; possibly the rhizomorphs of this latter fungus can enable a certain amount of air to reach the mycelium in the waterlogged areas.

(5) *Enzymes of wood-rotting fungi.* In comparison with the enzymes of other organisms those of the wood-rotting fungi have received but little systematic investigation and much of the information about them is of a very general nature. Most of the earlier workers were content to test an extract from the fungus against various substrata, and if decomposition of these substances was achieved the existence of a specific enzyme was assumed and an appropriate name was coined for the supposed enzyme. For instance, if lignin was found to be attacked the presence of a specific ligninase was presumed, in spite of the fact that all the analytical evidence has gone to show that decomposition of lignin by those fungi seems to be associated with intense oxidase activity (see p. 152).

Some of the difficulties experienced by the earlier investigators were probably due to the fact that they worked principally with extracts from the fruit bodies. It has since been shown that the enzymes in the sporophore are usually different in nature and present in smaller quantities than in the vegetative mycelium. Other workers used extracts from old cultures, in which enzyme activity had probably passed its peak.

Another obstacle to progress in the study of these enzymes has been the difficulty of obtaining the substrates on which they are to be tested in a physico-chemical state similar to that in which they occur in the wood. Some forms of chemically prepared cellulose evidently differ considerably from the material which occurs in the tree, for fungi which grow readily on wood make but poor growth on filter-paper cellulose, even when the other necessary nutrients are added. The same is no doubt true to an even greater degree about the so-called 'pure' lignin which is extracted from wood. This appears to be highly resistant to decomposition by organisms which are known to be able to attack it when it is present in the naturally occurring ligno-cellulose complex.

Nutman (1929) examined the enzymes present in an extract from young, actively growing mycelium of *Polyporus hispidus*, and found evidence for the presence of emulsin, diastase, invertase, ligninase, hemicellulase, oxidase and catalase, but he himself did not consider this list to be exhaustive. Findlay (1932) found that hydrolysing enzymes, but no evidence of oxidizing ones, were present in *Paxillus panuoides*. Montgomery (1936) examined the enzymes of *Fomes fraxineus* (which causes a white rot) and found oxidizing enzymes to be more abundant than those of a hydrolysing nature.

The most recent and comprehensive study of the enzymes of wood-rotting fungi is that of Bose & Sarker (1937), who studied the activity of both the intracellular and extracellular enzymes in eight species of Polyporaceae at three different stages of their growth. They found that the amount of extracellular enzymes was in all cases much larger than that of the corresponding intracellular ones, indicating that the major portion of the enzymes formed in the cell is secreted externally into the medium. On a priori grounds this might have been expected, and it is somewhat remarkable that so little attention had been paid by previous workers to the enzymes secreted into the surrounding medium by the fungi. Bose & Sarkar reported that with the exception of catalase, the activity of all the enzymes tested was greater in mycelium in the vegetative state than in that which was fruiting or about to fruit. In addition to a number of enzymes capable of decomposing polysaccharides and lignin, they found small amounts of lipolytic and proteolytic enzymes. Of the Polypores which they tested, *Polyporus ostreiformis* was found to be the most active.

There is room for a great deal more research on the enzymes of wood-rotting fungi, both as to the nature of the enzymes and their mode of action, as well as on their occurrence in various fungi at different stages of their growth, and it is to be hoped that when this study is undertaken it will be carried out in conjunction with accurate microchemical investigations of the progressive decomposition of cell wall substance by the action of the fungi concerned.

(6) *Metabolic products of wood-rotting fungi.* The biochemical activities of some of the common moulds, e.g. *Aspergillus niger*, have been exhaustively studied,

but until recently little attention has been paid to those of the higher fungi. The production of relatively large amounts of organic acids by some species has already been noted (p. 148). The ability of a basidiomycete, *Schizophyllum commune*, to methylate sulphur with the production of methyl mercaptan has recently been demonstrated by Birkinshaw, Findlay & Webb (1942). It has long been suspected that wood-rotting fungi may be able to methylate arsenic with the production of volatile organic arsenical compounds in a similar manner to that of *Penicillium brevicaule*, for a garlic odour is produced when certain wood fungi are grown on a medium containing traces of arsenic, but so far such products have not in the case of the wood-rotting species been definitely identified.

Elementary analyses of the sporophores of many of the higher Basidiomycetes were carried out at an early date, and in Zellner's monograph (1907) there is an excellent survey of the work up to that time. Extracts from fruit bodies of the higher fungi have been used but little for pharmaceutical purposes, though *Fomes officinalis* (*F. laricis*), the quinine fungus, which contains a very high percentage of a bitter, resin-like substance, was formerly in considerable demand. So far the search for bacteriostatic substances does not appear to have revealed the presence of any suitable substances among the metabolic products of wood-rotting fungi.

VI. PHYSICAL EFFECTS OF DECAY IN WOOD

(1) *Effect on density*. Fungal decay soon brings about a reduction in the density of wood, and the percentage loss in dry weight brought about by a fungus is a useful measure by which the amount of decay which has taken place can be expressed quantitatively. This loss in weight is the result of the respiration of the fungus. The progressive loss in dry weight of samples of wood undergoing decomposition by a pure culture of a fungus has been followed by Findlay (1940*b*), who has shown that in the case of a fungus which causes a white rot in which all the constituents of the wood are attacked, this may proceed until the wood substance is totally consumed, but where the fungus is unable to decompose lignin and brings about a so-called brown rot, the maximum loss in weight which it can cause is round about 70 % of the original dry weight. Gäumann (1930) has shown that at least in the earlier stages of decay the fungus decomposes chemically more wood substance than it is able to utilize, so that strictly speaking the loss in weight does not give a complete measure of the total change brought about by the fungus: since, however, it bears a direct relation to the amount of true decomposition and is a quantity which can readily be determined, it is still generally used in experimental work instead of chemical analysis, to estimate the degree of decomposition brought about by fungi.

Once a piece of wood is permeated with mycelium, and until the constituents from which the fungus is deriving its nourishment become depleted, loss in weight bears a direct relation to time during which fungus has been allowed to act, and a graph of loss in weight plotted against time of exposure is a straight line over the greater part of its length. Lightness in weight is often taken by practical inspectors of timber to indicate the presence of dote, but by itself it is an unreliable test, since abnormally light timber may be the result of growth conditions. Since, however, timber with a specific gravity abnormally low for the species is usually weak, its rejection may thus be justified.

(2) *Effect on mechanical properties*. It is, of course, a matter of common knowledge that decayed wood is softer and less strong than sound wood, but it is only in the last few years that accurate measurements have been made of the amount of depreciation in the strength properties at various stages of decay. Cartwright, Findlay, Chaplin & Campbell (1931) examined the strength of samples of Sitka spruce after various periods of exposure to *Trametes serialis* and showed that over 15 % loss in strength as measured by equivalent fibre stress at maximum load occurred after only two weeks' exposure to fungus. At this stage the wood showed very little external sign of discoloration or other change, nor had an appreciable loss in dry weight occurred. Chemical analysis, however, showed that an increased percentage of the wood was soluble in dilute alkali, indicating that breakage had occurred in some of the cellulose chains. The progressive increase in the alkali solubility in the case of this brown rot could be very closely correlated with the falling away in mechanical strength. When the fungus brings about a white rot the alkali solubility does not afford any indication of the extent of the attack. Cartwright, Campbell & Armstrong (1936) investigated the effect of *Polyporus hispidus* on ash wood, and they found that while the bending strength is slightly reduced in an early stage of fungal infection, it is the toughness or shock resistance which is most rapidly affected, a 20 % reduction in impact bending strength (toughness) being observed after only 2 weeks' exposure to fungus. After 12 weeks' exposure, the toughness of the wood had dropped to 10 % of its original value, while over 90 % of the original crushing strength remained. It is evident that this early reduction in bending strength must be due mainly to a shortening of the fibres in the wood. It was concluded that the loss in strength of ash wood caused by *P. hispidus* is caused mainly by depletion and alteration of the cellulose and its associated pentosans.

Scheffer, Wilson, Luxford & Hartley (1941) have also investigated the effect of various fungi on the strength of Sitka spruce. They found that in wood attacked by *P. schweinitzii* in which the specific

gravity had been reduced by 10%, the maximum crushing strength was down by 30% and the toughness was reduced by 95%, while in wood severely rotted by *Fomes* (*Trametes*) *pini* in which the specific gravity had been reduced by 30%, the reductions in crushing strength and toughness were 70% and 95% respectively, thus emphasizing how the different mechanical properties suffer different percentage reductions under the action of the same fungus. Scheffer (1936) carried out a comprehensive series of tests to determine the influence of *Polystictus versicolor* on the chemical and physical properties of the wood of *Liquidambar styraciflua*. Changes in the various properties of the wood were correlated wherever possible. He noted that the cell walls were uniformly thinned as a result of attack. He found that initially most of the strength properties were lowered in varying degree, more rapidly than the specific gravity, and that in the later stages of decay *all* the strength properties fell more rapidly than the specific gravity.

(3) *Effect of decay on moisture relations and calorific value.* It is a well-known fact that decayed wood absorbs liquid water and becomes waterlogged very much more rapidly than sound wood. Presumably the bore holes made by the fungal hyphae in the fibre walls permit the escape of the imprisoned air and the entry of the water. It has been noted (*Forest Products Research Annual Report*, 1935) that slight incipient decay by *Stereum sanguinolentum* greatly increases the penetration of creosote into Norway spruce.

The influence of decay on moisture absorption and retention was also studied in the course of Scheffer's tests, and he found that 'decayed wood was somewhat more responsive to atmospheric moisture changes than the sound wood, decidedly more susceptible to changes in water in liquid form, and much more capable of maintaining a substantially higher moisture content when intermittently exposed to liquid water'. This means that once a piece of wood is affected with decay the moisture conditions in it will remain favourable for fungus growth over longer periods than in a piece of sound wood exposed to the same cycle of conditions. Scheffer (1936) also examined the loss in calorific value of the wood and found this to be directly proportional to the losses in wood substance which had occurred, there being no evidence of changes in the calorific value per unit weight of wood.

(4) *Effect on appearance and optical properties of wood.* Decay in wood is almost invariably accompanied by a change in colour: it may become bleached in the case of a white rot or darkened in the case of a brown rot. Scheffer (1936) has shown that the lightening in colour brought about in the early stages of attack by *Polystictus versicolor* is due to the destruction by the fungus of pigmented materials in the wood rather than to removal of lignin as was formerly believed.

The first effect of a white rot may be a darkening of the wood, e.g. the incipient stage of attack by *Stereum spadiceum* in oak is the formation of brownish streaks, and it is only in later stages that pipes of whitish rot appear. It is probable that this initial darkening is due to the oxidation of tannins by the enzymes of the fungus.

In some instances discoloration of wood may be due to the production of definite colouring substances by fungi, for example, the vivid green colour brought about by *Chlorosplenium aeruginosum* has been shown to be due to the production by this fungus of a substance which has been named xylindein by Kögl & Tauffenbach (1925). Similarly, the rich brown colour of brown oak is probably due to the production of a coloured product by *Fistulina hepatica* (Cartwright, 1937). The discoloration of wood which is commonly known as sap stain or blue stain is, on the other hand, due to the presence of dark-coloured hyphae of certain Ascomycetes (especially *Ophiostoma* spp.) in the cells of the wood and not to the secretion of any colouring matter by these fungi.

Lohwag (1937) has proposed the use of polarized light for the microscopic investigation of decayed wood, since cellulose is doubly refractive and visible between crossed nicols, while the anisotropic lignin and pectic substances are not. This method is probably of limited application, for the differences observed are conspicuous only in the later stages of attack.

Wood severely rotted by certain fungi, in particular by *Armillaria mellea*, may become strongly luminescent, but it is probable that this luminescence is emitted by the fungal hyphae and the wood substance itself is not actually rendered luminescent.

The change in the X-ray patterns obtained has been proposed by Schulze, Theden & Vaupel (1937) as a means by which the degradation of the cellulose in wood may be followed. In wood severely rotted by a brown rot the X-ray pattern of cellulose may entirely disappear after 6 months' exposure to fungal attack. X-ray photographs to detect areas of decay in poles have already been used under practical conditions.

VII. THE CHEMICAL EFFECTS OF DECAY IN WOOD

Decomposition of wood by fungi is of two types, which have been described as brown rots and white rots respectively. In brown rots the cellulose and its associated pentosans are attacked while the lignin is left in a more or less unchanged condition; in a 'white' rot all the components of the wood, including the lignin are decomposed. Classification of decay types along these lines was suggested by Falck & Haag (1927), Wehmer (1927) and Bavendamm (1927), who proposed the terms 'destruction rot' and 'corrosion rot' for the two types, the former covering the 'brown rots' and the latter the 'white rots'. Most of the earlier workers carried out their analyses on samples of wood in which decay had reached a fairly

advanced stage under natural conditions. In order to get an accurate picture of what is happening during the decay process, it is necessary to have available for comparison analyses of the original wood and to know how much wood substance has been lost during the progress of the decay: otherwise it is impossible to determine how much depletion each of the constituents has undergone. For instance, if a fungus utilizes all the constituents of the wood, it is possible that analysis, even of severely decayed wood, may reveal comparatively little change in the relative proportions of the components. Analytical results should, therefore, always be related to the original sound wood. Campbell & Booth (1929), working with Sitka spruce sawdust undergoing decomposition by *Trametes serialis* in pure culture, were able to show that the brown rot caused by this fungus was very similar in its effect to that of mild acid hydrolysis. In this brown rot the cellulose and its associated polysaccharides were depleted, while the slight loss in lignin could be accounted for by the fall in the methoxyl content.

On account of the bleaching action of some of the white rot fungi it had long been assumed that they attacked mainly the lignin in the wood, but more recent work has shown that while the fungi which cause a 'white' rot do decompose lignin, other substances are attacked at the same time, and no wood-rotting fungus has been described as capable of deriving *all* its energy from the lignin in wood, and indeed it would be surprising if such should be found, for lignin is a stable and comparatively inert material which is resistant to decomposition. Lutz (1930) followed the degradation of beech wood by *Polystictus versicolor* using micro-chemical staining methods for the recognition of the products of decay, and summed up the process as a typical hydrolysis, successively decomposing the lignin, the cellulose, and finally the middle lamella, the cell wall substance being degraded into insoluble gums, then to soluble gums, and finally to sugars, which constitute the source of carbon for the nutrition of the fungus. Campbell (1931, 1932) examined the chemical action on beech wood of various other white rot fungi, and endeavoured to classify them into three groups, according to which constituents of the wood are attacked first. There is fairly good evidence for assuming that the intensity of the attack on the lignin is directly related to the oxidase activity of the fungus: for instance, an extract from the mycelium or culture fluid of *P. versicolor*, which can cause an active white rot, gives a very strong reaction when tested with guaiacum solution for the presence of oxidases. Bavendamm (1928) has described a test by which the presence of the oxidases characteristic of the fungi which cause white rots can be detected by growing the fungi on an agar medium containing tannin. If an oxidase is produced, a dark ring appears around the culture. The reaction of a large number of species of wood-rotting fungi has since been tested in this way by Davidson, Campbell & Blaisdell (1938). They found that 156 species, representing 96 % of those associated with white rots, gave positive reactions, the intensity of the reaction showing considerable variation. A few of the fungi associated with brown carbonizing rots gave inconsistent results, but in most of those tested, no reaction was obtained on the gallic or tannic acid medium.

Campbell (1932) suggests that one of the principal chemical bases for differentiating between white and brown rots is the difference in the effect on the alkali solubility, which in the case of a brown rot steadily increases as decay progresses, and is roughly proportional to the amount of cellulose depletion, while in a white rot it may arise slightly at first, but soon falls back to or below the original figure for sound wood. Wiertelak (1932) examined the effect of decay caused by *Trametes pini* and *Polystictus hirsutus* on sawdust of *Pinus taeda* and *Abies concolor*, and found that while in general the white rot results in a marked decrease of the lignin, a slow consumption of the cellulose goes on, preceded by its gradual chemical modification: *Polystictus hirsutus* being able to attack cellulose more rapidly than *Trametes pini*.

Scheffer (1936), in his comprehensive study of the effect of *Polystictus versicolor* on red gum sapwood, followed changes in its chemical composition as decay proceeded. He found that the relative proportions of the principal wood components were not materially altered. Based on the weight of the original sound wood, the lignin, pentosans not in the cellulose, cold-water soluble and 1 % alkali-soluble components were first attacked, while the Cross & Bevan cellulose, pentosans in cellulose and strictly hot-water-soluble portions were little affected until part of the wood substance had been consumed. In the earlier stages of decay the depletion of cellulose was represented by losses in both the stable and unstable forms, while in later stages the consumption of stable cellulose was more pronounced.

The general mode of action of the two types of decay—the brown and the white rot—are thus now fairly clear, and a general picture of the process of fungal decay in wood can be built up, but as yet comparatively little information is available about the intermediate products formed during decomposition. The successive degradation of cellulose and of the other polysaccharides in the wood appears to begin with a shortening of the cellulose chains, and this, with increasing hydrolysis of the polysaccharides, leads to the breakdown of the condensation products of the various wood sugars with formation of the corresponding hexoses and pentoses, which at certain stages in decay are formed more rapidly than the fungus can utilize them, so that an extract from decayed wood shows strongly reducing properties when tested with Fehling's solution. Intensive research into the nature of the lignin complex is now being undertaken, and when the structure of this substance is better understood it will be possible to find out how closely the residual lignin left after

fungal decay resembles the original material as it occurs in the cell walls of the wood.

By the use of microchemical methods, which will enable small samples to be examined, it should eventually be possible to obtain fundamental information about the composition and arrangement of the principal cell wall components by following the changes which take place as breakdown caused by fungal decay proceeds. In this connexion one may regard the enzymes of the fungus as mild chemical reagents which can be used under controlled conditions to bring about less drastic changes than those wrought by the reagents such as 72 % sulphuric acid, which are commonly used in wood analysis.

VIII. EFFECT OF FUNGAL DECAY ON MICROSCOPIC STRUCTURE OF WOOD

Reference has been made (p. 145) to the comparatively slight diagnostic value of the appearance of fungal hyphae in wood and to their effect on the cell walls. While wood-rotting fungi differ in the extent to which they penetrate the cell walls, their action within the two groups of the brown and the white rots appears in the main to be similar.

In wood attacked by a fungus which causes a brown rot, the cell wall does not appear to be thinned appreciably until a very late stage of decay, and even when practically all the cellulose lattice has been removed, the residual lignin preserves the form of the original cell walls. It is characteristic of most of the active brown rots that the hyphae which penetrate the walls form bore holes, which become appreciably wider than the diameter of the hypha. In white rots, on the other hand, a general thinning of the cell walls, in addition to the formation of bore holes, is a usual feature of the more advanced stage of decay. This is rather to be expected, since all the constituents of the wood are decomposed by white rot fungi. However, the residual cellulose left in wood attacked by white rot is sufficient, even in a quite advanced stage of rot (e.g. up to 70 % loss in weight), to enable the wood to retain its shape and outward structure, whereas in a brown rot disintegration due to removal of cellulose begins to appear at a much earlier stage of decay. The penetration of membranes such as cell walls by fungal hyphae is a problem which has attracted the attention of a number of workers. Nutman (1929) described the penetration of the cell wall in ash by hyphae of *Polyporus hispidus* as follows: a young hypha, so fine that it appears merely as a line of protoplasmic granules under $\frac{1}{12}$ in. objective, comes in contact with a wall. It bores straight through, usually at right angles to the wall, with no change of diameter. Nutman suggests that at this stage the penetration must be enzymatic, since it would appear difficult for so fine a thread with no appressorium to penetrate a thick lignified cell wall by mechanical means. After penetration the young hypha thickens until it reaches its mature size (i.e. in this case 1·5 μ), while the portion in the wall remains exceedingly fine. After this erosion starts from one end of the thin thread and is soon followed by erosion at the other end; this continues until two cone-shaped openings are formed with their axis along the hypha. As the cones increase in size the hypha swells up. Eventually with this species a large cylindrical or hour-glass-shaped bore hole, with a diameter several times that of the hypha, is formed, in which the hypha lies unconstricted. The amount of subsequent enlargement which takes place varies greatly from species to species, and with some fungi the bore holes remain small, with the hypha always showing a constriction where it passes through the cell wall.

Cartwright (1930), who studied the penetration of the cell walls in spruce by the hyphae of *Trametes serialis*, observed that on approaching or on contact with a tracheid wall the contents of a small hypha become concentrated at the tip, from which a fine projection emerges; this develops into a fine thread, which on contact with the wall forms a V-shaped nick. He concluded that the actual penetration must be rapid, since it was extremely rare to find cases in which the hypha had penetrated only partly through a wall. He noted that with this species the bore holes continued to enlarge after the penetration of the wall and that sometimes it might reach a size several times greater than that of the hypha. He came to the conclusion that the cellulose in the cell wall must be altered chemically by the enzymes of the fungus prior to penetration of the wall by the hypha, which might then be at least partly mechanical. Proctor (1941) investigated the mode of penetration of cell walls of four different woods by six species of fungi. He used very high magnifications and took every precaution to obtain preparations showing undistorted hyphal penetrations. His visual observations were supplemented by excellent photomicrographs taken with ultra-violet light. A large number of bore holes were also examined under polarized light, but no interference patterns of any kind that might indicate the presence of mechanical failures were observed. He finally concludes that penetration of the walls of wood cells is accomplished by (1) the secretion of enzymes at the tips of penetrating hyphae, and (2) the total local dissolution of the cell wall by enzymic activity in advance of actual passage through the wall. He thus postulates that penetration is effected through a preformed passage without actual contact between the hypha and the cell wall, except perhaps at the very first point of penetration, when a stimulus to initiate enzymic activity may be set up. He found no evidence for any existence of any mechanical forces being exerted during penetration. It may be noted in this connexion that the hyphae of sap-stain fungi, e.g. *Ophiostoma* spp., which penetrate the tracheid walls quite freely without any appreciable softening by chemical action having taken place previously, develop well-marked appressoria, so it is reasonable to imagine that the final stage of penetration by the

hypha of wood-rotting fungi through a wall already softened by enzyme action may involve some mechanical pressure, though the amount of force required may not be great enough to leave any evidence on the cell walls.

Tamblyn (1937) has described an unusual type of hyphal penetration in jarrah (*Eucalyptus marginata*). In this wood affected with so-called black-straw rot (caused by a basidiomycete fungus), large bore holes occur, which run for long distances down the secondary fibre walls *parallel to the long axis of the fibres*. These bore holes may be so numerous as to be almost contiguous in transverse section.

Bailey & Vestal (1937) have described peculiar cavities which are formed in the cell walls of a wide variety of trees by certain unidentified fungi, which are stated probably to be Pyrenomycetes. These cavities, which may be cylindrical or biconical in shape, are of remarkably constant angularity, regardless of the species of tree in which they occur, and in outline they recall the shape of certain crystals. Their formation suggests that hydrolysis of the cellulose through enzymatic activity proceeds along two clearly defined sets of planes: (1) those oriented parallel to the long axis of the fibrils and chain molecules of cellulose, and (2) those at an angle of 20–25° to this axis. It is much to be hoped that it will be found possible to isolate and identify these fungi, since a detailed chemical and physical study of their action on the cell wall may elucidate many problems relating to its physico-chemical fine structure as well as to the mode of action of the specialized fungi concerned.

IX. NATURAL DURABILITY AND RESISTANCE TO DECAY OF TIMBERS

Timbers vary enormously in their resistance to fungal decay, some, like sycamore and poplar, rapidly disintegrate after only a few months' exposure to fungal attack; others, such as oak and teak, will endure for very many years even when they are in contact with the soil and exposed to severe risk of fungal attack. Naturally the reasons for this difference have long been a subject of enquiry. Once a tree has been felled the heartwood in those species which show a well-defined heartwood is invariably more resistant to decay than the sapwood, and it was therefore concluded that it is the presence in the wood of those substances which are formed when the living cells in the sapwood lose their vitality and when heartwood formation takes place, that confers on it the resistance to fungal decay. In many species the amount of material which can be extracted by hot water is much higher in the heartwood than in the sapwood, and it was to these so-called extractives that attention was first directed. Hawley, Fleck & Richards (1924), who tested the toxicity to *Fomes annosus* of standard hot and cold water extracts prepared from a wide range of timbers, were able to show a close correlation between the toxicity of the extracts and the resistance to decay of the timbers from which they had been prepared; for instance, the hot water extract from such durable timbers as mulberry, catalpa and redwood (*Sequoia sempervirens*) entirely inhibited the growth of the test fungus. Anderson (1931) examined the toxicity of extracts of *Pinus ponderosa* to *Lenzites saepiaria*, but did not find the extract from the heartwood to be much more toxic than that from the sapwood. Anderson & Sherard (1933) described the isolation from *Thuja plicata* wood of an exceedingly toxic crystalline substance which they named dehydroperillic acid and for which a structural formula was proposed. Migita (1932) examined the resistance to decay of sawdust from four timbers after extraction with various solvents and concluded that the durability of *Thujopsis dolabrata* is due to the toxicity to fungi of its alcohol-benzene-soluble constituent (probably an ethereal oil), while that of *Castanea crenata* results from a similar property in its hot-water-soluble components. Ernest (1936) described a simple test by which she claimed it was possible to determine whether a timber contains a toxic substance soluble in water. One half of a small petri dish is filled with damp sawdust of the timber under examination and the other half with nutrient agar which is infected with a transplant of the test fungus. If the sawdust contains a toxic material soluble in water, this will diffuse into the agar and check the growth of the test fungus.

The important role of tannins in conferring resistance to decay has been pointed out by Lutz (1928), who showed that a fungus such as *Polystictus versicolor*, which normally does not attack oak heartwood, can be induced to do so if the wood is leached so as to remove the tannin. He considers that the tannin inhibits the oxidizing activity of the ferments produced by the fungus. It is noteworthy that those fungi which can attack oak heartwood are able to grow in media containing relatively high concentrations of tannin (Bavendamm, 1928).

Resistance to decay cannot be related directly to density. Although in a general way it is true to say that most of the very dense woods are durable, yet some comparatively light woods, such as western red cedar (*Thuja plicata*), are highly resistant to decay. It is probably true to say that in general the most durable timbers are those which have a high density combined with a high content of extractives toxic to fungi. There is a need for further research on the nature of the toxic extractives present in wood, and it has often been suggested that useful ideas for the development of new and more effective wood preservatives could be obtained were there a better understanding of the nature of the natural preservative material in durable woods.

The natural resistance to decay of timbers has been tested in the laboratory by a number of investigators, e.g. Humphrey (1915, 1916), Schmitz & Daniells (1921), Hubert (1929), Lutz (1935), Liese (1937), Findlay (1938). In general the methods have involved

the exposure of small sterilized samples of the timber to the attack of a number of test fungi growing in pure culture under controlled conditions of temperature, etc., and the amount of decay resulting from the fungal attack has been measured by estimating the loss in dry weight. The results of such tests have been found in general to agree quite well with the behaviour of the timbers in practice, and they are useful for enabling an opinion as to the probable durability of a new species to be given in a comparatively short time or to determine the susceptibility of some individual timber to a particular fungus, such as *Merulius lacrymans*. Field tests, usually known as 'graveyard' tests, have been carried out at a number of centres, and these are especially useful in tropical countries where resistance to termites is equally as important as resistance to fungi, for it is very difficult to carry out tests in the laboratory on the relative susceptibility of timbers to termite attack. Such 'graveyard' tests have recently been carried out by officers of the Forest Departments in many tropical countries, and progress reports on the result obtained have been published in the annual reports of the various Forest Departments concerned. Only rarely has any attempt been made to list the fungi which develop on the test pieces or to keep any account of the fungal flora which develops at various stages during the 'life' of the test sample. Such information would have considerable interest from an ecological point of view, for it is obvious that in nature a log of wood suffers attack from a definite succession of forms. Brooks, Adamson, Baker & Crowdy (1941) have listed the fungi which appeared on the test pieces in graveyard tests carried out in Trinidad, and it is to be hoped that similar information will become available from other localities.

Great variation in the resistance to decay occurs within the same species of timber. In coniferous timbers slow-grown timber from mature trees tends to be more resistant than wide-ringed material from young trees, but resistance to decay does not appear to be correlated with ring width or density to anything like the same extent as do the strength properties of the timber. Findlay (1940*a*) examined the resistance to decay of *Pinus sylvestris* from a number of different sources and found that Russian material was only slightly more resistant to decay than that from more southerly countries. Cartwright (1941) has shown that considerable variation may exist within a single log of *Thuja plicata*, the most durable timber being that derived from the outer heartwood adjacent to the sapwood in the butt of the log. It is possible that the comparatively low resistance of the heartwood near the pith of the tree may be the reason why butt rot almost invariably makes its appearance at the centre of the trunk and slowly works its way outwards, but further tests with other species of trees will be necessary before this suggestion can be applied as an explanation of the form taken by butt rot of trees in general.

It is a well-established belief among timber merchants, that timber felled in the spring and summer is less resistant to decay than timber felled in autumn and winter. Gaümann (1930) carried out a large number of carefully controlled experiments in order to discover whether this belief has any foundation in fact. He concluded that while the external factors of climate and the greater intensity of infection in the summer account for much of the deterioration which occurs in summer-felled timber, yet there is in spruce and fir, while still in the green, unseasoned condition, an actual inherent difference in resistance to decay, the wood felled in the early summer being more rapidly decayed, under controlled conditions, by the test fungi which were used than wood felled in winter. These differences were more pronounced in the sapwood than in the heartwood, but nevertheless were shown to exist in the latter. He carried out an exhaustive series of analyses to determine the content of the various substances in the wood at different times of the year, but was unable to correlate the change in resistance to decay with any fluctuation in any of the major constituents of the wood, and in consequence he concluded that the reason for difference in resistance is to be found in variations in the physico-chemical structure of the cell wall at different seasons, rather than in alterations in the chemical composition of the wood. After seasoning for one year in the open, the difference in decay resistance between the summer- and the winter-felled timber was found to be very much less than that found when the timber was tested in the green condition, and this fact tends to support Gaümann's view that it is the physical condition of the colloids in the cell wall which is the factor responsible for the differences observed.

Since timber is not usually used until it has been seasoned, it would appear that there is very little risk in using summer-felled material, provided, of course, that it can be seasoned without serious degrade owing to cracking and blue-stain, both of which are more liable to occur under summer conditions than during the colder months of the year. Gaümann (1938) did not consider that there was any significant difference in rot resistance of beech felled at different seasons. In view of the very pronounced views held by practical men on this subject, it would be of interest to investigate this problem further and to include tests on a range of species, both broadleaved and coniferous.

X. PRESERVATION OF TIMBER BY CHEMICAL TREATMENT

While the technical aspects of timber preservation are outside the scope of this review, it may be of interest to describe briefly some of the progress that has been made in recent years.

Coal tar creosote has been found so effective a preservative for timber that it still holds the field after

nearly a century of use, for the preservation of structural timber which is exposed to the weather or used in contact with the soil. A great deal of work has been carried out in order to determine the relative effectiveness of the various components of creosote oil, and recent research tends to show that the highly toxic tar acids do not play such an important part as they were at one time believed to do.

The use of water-soluble preservatives has tended to increase since the discovery that by the addition of chromate to solutions containing arsenic, zinc or mercury in a soluble form, these elements become 'fixed' or rendered insoluble in the wood after it has been impregnated, so that the treated wood can safely be used in situations where it may sometimes be exposed to the leaching action of rain. Many of the most successful mixtures of salts for the preservation of wood are based on variations of a mixture proposed by Malenkovic which included sodium fluoride and dinitrophenol or its sodium salt. The files of the Patent Offices contain endless descriptions of various preservatives made up of various combinations of salts toxic to fungi, but the vast majority of these have never been developed. In recent years a number of oil-soluble preservatives consisting of a toxic substance such as copper naphthenate or pentachlorphenol dissolved in white spirit has been put on the market. This type of preservative has certain advantages for the preservation of timber on which it is desired to apply subsequently some decorative finish and when any distortion of the ready shaped timbers is undesirable, e.g. for the preservation of joinery, of the wooden parts of motor bodies, and of the wooden parts of boats such as yachts and lifeboats, etc.

The need was soon felt for some agreed method of testing wood preservatives in the laboratory, so that results obtained in one research institute could be directly compared with results obtained in other similar places. In the U.S.A. the so-called agar or Petri-dish method was used almost exclusively until a few years ago. By means of this method, which consists in determining the minimum quantity of the substance which incorporated into a nutrient agar medium prevents all growth of the test fungus thereon, it is possible to get repeatable results with some degree of accuracy. Most European workers have felt, however, that the physical conditions in an agar gel are so very different to those pertaining in wood that it is preferable to carry out tests with the substances impregnated into wood blocks which are subsequently exposed to fungal attack under controlled conditions. They have maintained that it is preferable from a practical point of view to get results which may be less precise, but which bear some relation to practical conditions. As a result of a conference held in Berlin in 1930, a standard method for carrying out tests to determine the toxicity to fungi of wood preservatives was described (Liese, Nowak, Peters & Rabanus, 1935). Since then a British Standard specification (1939) has been published, which gives details of a standard test which follows the general lines of that proposed by the international committee.

So far only limited success has been obtained in efforts which have been made to correlate toxic activity with chemical structure, but a great deal of useful information on this subject has been accumulated during a series of investigations which were carried out at the Forest Products Laboratory in Madison, with the object of developing effective fungicides for the surface treatment of lumber against moulds.

XI. FIELD STUDIES OF TIMBER DECAY AND THE APPLICATION OF THE RESULTS OF RESEARCH TO PRACTICE

Field studies of the occurrence and prevalence of various types of decay on timber in service have covered a wide range of localities in which timber is used. Dry rot in buildings has been studied in relation to the types of construction involved. Once the fundamental conditions for the growth of the causal organisms have been elucidated, application of this knowledge must be related to architectural design so that conditions suitable for fungus growth do not arise, or if they accidentally develop the timber is protected against attack. When, for instance, it has been established that *Merulius lacrymans* cannot develop at a moisture content below 20% of the weight of the wood, then it becomes the duty of the architect or builder to design his buildings so that under no condition will the moisture content of timber in a building exceed this figure. If experience shows that there is inevitably a risk of woodwork remaining moist over any length of time, then preservative treatment must be applied before the timber is put into service. It is now common practice to creosote under pressure all railway sleepers and telegraph poles, but even to-day a great deal of untreated fencing is erected, only to decay a few years later. As in so many other fields of human activity, the development of fundamental knowledge has far outstripped the application of scientific research to practice. The decay of timber in mines is a striking example of the failure to apply to practice the results of research. Cheap methods for the preservation of mining timber have been known for half a century or more, yet until a few years ago replacement of the timbering along the permanent ways in some damp coal mines has had to be undertaken almost yearly as a result of fungal decay. In the last few years there has been a great development of timber preservation in the South African gold mines, and the operating companies now find it worth their while to maintain a special laboratory for the study of timber problems.

All too often in the past the liability to decay of timber has been accepted as an inevitable defect in this, the most useful of all constructional materials,

and it is much to be hoped that in the future education of the prospective users of timber will enable them to utilize timber in such a way that subsequent losses through fungal decay are reduced to a minimum.

XII. SUMMARY

After a brief historical introduction, attention is drawn to the importance of the correct sylvicultural management of woodlands in the production of clear, disease-free timber. Methods for the identification of the organisms which cause decay in timber are discussed, and the importance of pure culture methods is stressed. The principal contributions to the general descriptive work on timber decay in various countries are referred to, and it is pointed out how the usefulness of much of this work has been enhanced by the publication of books and monographs collating this scattered information. In a discussion of the physiology of wood-rotting fungi the temperature relations and nutritional requirements, growth in relation to *p*H, and moisture content of the substratum are considered. The unsatisfactory state of present-day knowledge of the enzymes of wood-decaying fungi is mentioned. Recent work on the metabolic products of wood-rotting fungi is referred to briefly. The effects of decay on the physical properties of wood are described, i.e. on the density, strength and optical properties, etc. The mechanical strength of timber, especially the toughness, may be appreciably affected in a very early stage of decay before any measurable loss in weight has occurred. The chemical changes brought about during the decomposition of wood are discussed. Rot of timber can be classified chemically into two main types: brown rots, in which only hydrolysis of cellulose and other polysaccharides occurs, and white rots, in which all the constituents of the wood, including the lignin, are attacked in varying degrees and in which oxidizing as well as hydrolysing enzymes are present. The effect of fungal attack on the microscopic structure of wood is described, and the theories put forward to explain the penetration of the cell walls by hyphae are critically examined. Methods of determining the natural resistance to decay of timber in the laboratory are referred to, and the basis of natural durability in wood is shown to lie mainly in the nature of the chemical 'extractives' present in the wood. Recent advances are described in the use of water-soluble wood preservatives which become fixed in the wood by the addition of a chromate to the treating solution, and the establishment of a British Standard method of test and the use of field tests are noted. Finally, attention is drawn to the great lag in the application to practice of recent advances in the study of timber decay and its prevention.

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MINOR ELEMENTS AND PLANT GROWTH

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I. INTRODUCTION

Most of the scientific investigations on the practical questions dealing with plant nutrition can be said to date from 1843, when Lawes and Gilbert began their classical work at Rothamsted. In the early days attention was focused on the obvious soil nutrients, especially nitrogen, potash, phosphate, calcium, magnesium and iron. Since the beginning of this century it has become increasingly evident that other elements, present or available in the soil in small quantities only, also have a part to play in the economy of the plant. Claims were soon made that some of these elements were essential to growth, but at first no distinction was drawn between 'essential' and 'beneficial', and it remained for later workers to demonstrate how difficult it is to prove that an element is really essential, i.e. that in its absence the development of the plant cannot proceed normally, if at all. The quantities involved are often so minute that most elaborate precautions are necessary to insure the complete absence of any particular element from nutrient salts, water, and other sources of possible contamination. The earlier workers either failed to realize the efficiency of these traces of 'minor' elements or were not in a position to purify their materials sufficiently, and to this day there are relatively few cases in which the essential nature of a minor element can be regarded as fully proved by direct means. Many of the claims rest on indirect evidence, as when the provision of a small amount of a certain element encourages satisfactory growth of a crop which has hitherto failed on the area concerned.

The work on minor elements has caught the popular imagination and, important though it is, there is a danger that many plant ills that cannot be satisfactorily explained may be vaguely attributed to a deficiency of this or that element. Literally thousands of papers have been written on the subject and, in a review of this kind, it is manifestly impossible to do more than give an outline of the position, with references to a small proportion of the literature to illustrate the main points. Various bibliographies and reviews have been produced and should be consulted for further information on specific elements, particularly that originally compiled by Willis (1939–42).

During the last decade the focus of attention has shifted somewhat from the critical consideration of the essential nature of certain minor elements for plant nutrition (Brenchley, 1936) and more work has been done on the practical aspects of the problem. It is therefore proposed to deal mainly with this more recent work, widening the scope so as to include any information which bears on the economy of the minor elements in relation to the growth and the utilization of plants. The diagnosis of mineral deficiencies is normally laborious and frequently uncertain, but Roach (1940) and his collaborators (Hill & Roach, 1940) have devised methods of spectrographic and chemical analyses and plant injection whereby the mineral status of plants can be determined rapidly, and the disclosed deficiencies made good by injection or by soil treatment.

II. BORON

The essential part played by boron in plant growth was foreshadowed by Agulhon (1910), but definite proof was not forthcoming till Warington (1923) showed that in the absence of boron from the nutrient solution *Vicia faba* is unable to make much growth before necrosis of the meristematic tissues results in the stoppage of all growth. This was confirmed later (Brenchley & Warington, 1927) for various other plants, though the function of the minute quantity of boron required remained obscure. Much work all over the world, in the laboratory and in the field, established the fact that boron deficiency is the cause of various plant disorders which had hitherto been classed as 'physiological diseases', heart rot of sugar beet being an outstanding example. Plant after plant has been shown to need boron, and so much has been written on the subject that in the Rothamsted files, still incomplete, nearly two hundred species are recorded, many from at least six sources. It is impossible, from this point of view, to do more than direct attention to the two main bibliographies that attempt to cover all references to the boron question (Willis, 1939, etc.; American Potash Institute, 1939, etc.). Dennis & O'Brien (1937) also made an excellent résumé up to date on the question of boron in agriculture, with many classified references.

Although the main problem of the real function of boron in the plant economy still remains unsolved, various points have been established which may ultimately help in its solution. It is recognized that too much boron is definitely toxic (Hodgkins, Hageman & McHargue, 1942), some species being more sensitive than others. Boron carried by irrigation water can do much damage to such crops as lemon

(Eaton, 1935), as little as 0·5 part per million of boron being toxic, whereas datepalms and asparagus are much more resistant. Tomatoes are able to stand high salt concentrations and are not affected by large doses of boron (Fisher, 1935). When boron is used as a fertilizer there is little danger of its accumulation in the soil, as it is easily leached away (Krugel, 1938). Surface applications of boron may penetrate into the soil to a depth of 30 in., but in view of the loss by leaching, Askew *et al.* (1938) suggest that where necessary small annual applications may be more effective than larger treatments at longer intervals. An interesting suggestion was made by Edelman (1939) that in coastal areas soil deficient in boron may receive a valuable addition, to the extent of about 80 g. B_2O_3/ha., in the form of cyclic salt, i.e. drops of sea water picked up by the wind and deposited by snow and rain on the soil.

The amount of boron available to the plants is reduced by alkaline conditions and summer drought, and on soils with a low boron status crops may suffer from deficiency diseases in some years but not in others. Brandenburg (1939) made a comprehensive survey of the basis for the use of boron in agriculture, and concludes that boron must be considered to be as valuable as any other nutrient for the operation of metabolic processes in the plant, thus supporting the earlier work of McLean & Hughes (1936). The danger that overliming will induce boron deficiency in crops by rendering the soil boron unavailable is emphasized by various workers, most of whom also indicate the ameliorating effect of applications of borax (Guilbert, 1936; Naftel, 1937; Midgley & Dunklee, 1939). A possible correlation between the calcium and boron metabolism of the plant was suggested by Warington (1934) for *V. faba* but not confirmed by Holley & Dulin (1937) for cotton. Marsh & Shive (1941) and Marsh (1942) however, made critical experiments on maize and oats among the monocotyledons and tobacco and *V. faba* among the dicotyledons, and found a definite relation between the boron in active tissues and the pectin content, and between boron and fat metabolism in which calcium is involved. The soluble calcium in maize tissue is determined not by the total calcium in the plant, but by the boron content.

As a convenient source of boron supply the use of Chilean nitrate as a fertilizer has been much discussed, as it contains sufficient boron to satisfy the needs of crops. The work of Hudig & Lehr (1938), Scharrer & Schropp (1937*a*), Shive (1936), and Brenchley (1943) may be referred to in this connexion. One aspect of the use of boron in fertilizers needs more investigation, as there are conflicting opinions as to its effect upon the keeping quality of the harvested crops. For instance, Chittenden & Thomson (1938) found that apples from trees manured with borax failed to keep as well as those from control trees, and pointed out that on boron-deficient soils one-half pound of borax per tree is the maximum that can be used without significantly lowering the keeping quality of the fruit. On the other hand, Phillips (1938) claimed that with tomatoes moderate amounts of boron induced a steady low respiration rate, apparently conducive to good keeping quality.

The question of disease caused by lack of boron is bound up with the physiological and morphological reactions of the plant to such deficiency. Abnormal meristem reaction is characteristic (Warington, 1926; MacArthur, 1940), while disintegration or hypertrophy of the cells of various tissues, chlorosis, root tip injury and other symptoms are commonly manifested (Eltinge, 1936).

Chandler's work on the genus *Brassica* (1941) gives an excellent corroboration of the earlier results obtained by various investigators, specifically stating that all the signs of boron deficiency found in Brassicas are secondary expressions of the alteration and retardation of meristematic activity. One point of special interest is the assertion that plants may receive their boron through the leaves just as effectively as through the roots. The reproductive organs are much affected, as normally they may contain the heaviest concentration of boron within the plant (Bobko & Zerling, 1938). Blaha & Schmidt (1939) obtained a very heavy increase in the germination of pollen from fruit trees in a sugar medium plus a trace of borax. Sugar beet affected by boron deficiency in the field will push out healthy lateral shoots and produce normal seed if an adequate supply of boron is provided for the second year's growth (Brenchley & Watson, 1937). Similar recovery has also been obtained in carrot (Warington, 1940).

In spite of all the work on boron very little definite knowledge is yet available as to the actual part played by the element. The absorbed boron does not appear to be translocated from one part of the plant to another as growth proceeds, as if the boron supply is cut off during growth the older organs retain their normal boron content while the younger tissues exhibit signs of deficiency (Kentucky Exp. Sta. 1936). Though it is generally accepted that boron must have some definite function within the plant, there have been suggestions that the action is indirect, owing to its effect upon the intake of other nutrients (Rehm, 1937). Again, Schmidt (1937) put forward the idea that boron acts by reducing the permeability of the root protoplasm in the presence of excess nitrogen in the soil, claiming that even on boron-deficient soils heart rot of sugar beet did not occur if nitrogenous manuring was at a low level.

One other function of boron that may be mentioned is concerned with the development of nodule bacteria on the roots of leguminous plants. Brenchley & Thornton (1925) showed the failure of nodules to develop normally on *V. faba* in the absence of boron, thus reducing the usual accessory supply of nitrogen for the plants. Similar results were obtained by Lebedev (1940) with lupins, in which boron stimulated the development of the nodule bacteria and had a beneficial effect on the growth of hemp grown in

association with lupins, hemp utilizing the nitrogen fixed in the nodules.

III. COPPER

Most of the earlier work on the relation of copper to plants was concentrated on its toxicity, the more so since this property rendered it invaluable as a means of controlling certain fungal diseases, as *Phytophthora infestans* on potatoes and tomatoes and *Peronospora infestans* on vines. Later, it was found that on certain soils the addition of copper salts was vital to crop production (Allison, Bryan & Hunter, 1927), though the mechanism of its action remained obscure. Claims were also made that traces of copper are essential to the proper development of plants, but here again the evidence remained nebulous and contradictory, with doubts whether any beneficial action observed was direct or indirect.

During the last decade much work has been done on the element, chiefly from the practical point of view, in connexion with crop improvement. Some work has dealt with the physiological side of its possible function as an essential plant nutrient, but even now the point cannot be regarded as fully proved as in the case of boron.

It has repeatedly been confirmed that when attempts are made to bring under the plough peat, or other highly organic soils, such as the 'muck' soils of America, the crops are liable to suffer from 'reclamation disease' unless a copper salt, usually sulphate, is supplied as an ameliorating agent. Associated with these results are many claims that copper is an essential plant nutrient, but still the evidence is insufficient to make it certain whether the improvement occurs within or without the plant, i.e. whether the copper is actually necessary for vital physiological functions or for building up plant tissues, or whether it acts on the soil, releasing or rendering available other plant nutrients, altering the physical constitution of the soil or influencing the movement of water in the soil (Arnd & Segeberg, 1936). For practical purposes this is probably unimportant, but from the wealth of observations that is accumulating the truth may ultimately emerge.

The various workers on the problem of reclamation disease, under its local names, are unanimous with regard to the efficiency of copper in improving matters, though the angles of approach differ greatly. Many affected soils are definitely short of copper (Vinogradov, 1940; Hoffmann, 1939), but Rademacher (1936) indicated that copper is not necessarily absent from affected soils, but that possibly it is rendered unavailable by such substances as lignin and humic acid. The quantity of copper sulphate required for amelioration is connected with the humus content of the soil, as soils rich in humus require much more additional copper than those containing little humus (Meijer, 1934). Soil analyses show that available copper is at a minimum at a *p*H between 5·5 and 6·5 (Steenbjerg, 1940).

Adequate manuring is essential for the copper dressing to be fully effective (Rademacher, 1935; Husemann, 1937), as in some cases poor growth after treatment may be caused by the heavy demands of a crop for some particular fertilizer as when spinach follows carrots, both absorbing much potash (Knott, 1938). Some strains of cereals, as oats, prove more resistant to reclamation (or heath moor) disease because of their greater ability to absorb copper from poor soil (Rademacher, 1937). Finely ground copper refinery slag, hitherto a waste product, containing 0·5% copper oxide, was found to be as effective as copper sulphate (Nicolaisen, Seelbach & Leitzke, 1939), the amount required being from 273–819 lb./acre. Some investigators claim that the function of the added copper in peat soils is to serve as an agent for decreasing the availability of iron and manganese, where these are present in abnormally large amounts (Willis & Piland, 1936; Lazarev, 1939).

In connexion with all this work on reclamation disease the specific action of copper on many plants has emerged. The reaction of different species varies, and may not always be the same on different soils or in different circumstances. Diseased conditions manifest themselves in various ways; growth is poor, and many plants die without reaching maturity, chlorosis of the leaf tips is very usual, as in 'white-tip disease' of barley and oats (Steenbjerg, 1940), and reproductive processes are inhibited. In tomatoes copper deficiency causes dwarfing, curling of leaflets, colour changes and necrosis resulting from the separation and shrinkage of the palisade cells of the leaves (Reed, 1939). Copper is said to be vitally concerned with the production of pollen in maize and to affect flower formation in tomatoes and geraniums (Kentucky, 1934, 1935). Nodules on lima-bean roots were doubled in number when copper sulphate was applied, though the largest nodules occurred where copper was not supplied (Manns, Churchman & Manns, 1936). In Holland, Mulder (1938*a*) has written in much detail on reclamation disease and its causes, and points out that wheat plants deficient in copper are more susceptible to diseases of the ear than are normal plants (1940). Also, the colour and appearance of *Aspergillus niger* can be used as a means of determining the presence of very small amounts of copper in soils (Mulder, 1937, 1938*b*, 1939) and so judging whether or not a soil is liable to reclamation disease.

On affected land cereals, especially oats, tend to produce empty heads, which fill out if copper is supplied (Arnd & Hoffmann, 1937; Undenas, 1937; Nicolaisen & Seelbach, 1938). In field and pot experiments Rademacher (1940) found that for oats the period of maximum copper requirement is in the early stages, prior to flowering, and that if the necessary minimum is not obtained early a later supply will not ensure normal development of seed. In wheat Prescott (1939) states that improved yield with copper is due to increases in the number of heads per plant,

in the number of grains per head and also in the average weight of individual grains. Calcareous sand dunes in Australia, on which animals are liable to 'coasty disease', will grow rye satisfactorily (Piper, 1938), but other cereals fail unless copper is applied (Riceman, Donald & Piper, 1938; Riceman, Donald & Evans, 1940). Traces of copper were found to improve the germinating power of rice, the yield of grain also increasing with some low concentrations (Tokuoka & Morooka, 1937).

Sugar beet (Proskura, 1940; Van Schreven, 1936*a*), beans (Townsend, 1939), tobacco, cotton (Churchman, Manns & Manns, 1937), onions (Knott, 1936), lettuces (Wilson & Townsend, 1933), vines and maize (Vasileu, Huber, Pantea & Timosencu, 1937, 1938) are among the various crops found to benefit by copper manuring in areas where they normally do not thrive. In the case of potatoes, interest largely centres round the effect of spraying upon the yield and cooking quality of the crop, and the evidence is conflicting, probably because of the wide range of soils from which the reports come. Apart from protection from loss due to blight, there are claims that available copper increases the yield, weight and number of tubers when the crop is lifted (Teakle & Stewart, 1939), though there may be retardation in tuber development at first after early spraying (Mader & Blodgett, 1935). Tubers from sprayed plants showed less blackening on cooking than those from unsprayed plants (Mader & Mader, 1937; Smith & Nash, 1937). In other cases no increase in potato yields was obtained where copper had accumulated in the soil from continuous spraying with Bordeaux mixture over a period of years (Skaptason, Peterson & Blodgett, 1940). In citrus orchards it has been noticed that trees receiving an additional combination of copper, zinc, magnesium, and manganese were distinctly more resistant to cold than those with a straightforward N.P.K. treatment (Lawless & Camp, 1940). Exanthema or 'die-back' of citrus has been successfully treated by applying copper sulphate to the soil, helping to decrease the abnormal quantity of nitrogen present in the affected tissues (Fudge, 1937). The same results have been obtained by spraying with Bordeaux mixture (Haas & Quayle, 1935). Exanthema on Bartlett pear trees has been cured by administration of copper salts directly to the root crown of the trees as well as by spraying or by direct application to the soil (Oserkowsky & Thomas, 1938).

Scattered observations exist on the copper content of plants at various stages of growth. In tomatoes a progressive increase in copper is recorded with the maturation of the fruits, the amount also varying with the physical condition and *p*H of the soil, increasing on the whole with coarse soils and low *p*H values (Weber & McLean, 1938). In rice the copper content of the root, stalk and leaf are greatest during the periods of rapid growth and seed production (Sarata, 1938).

Experiments in nutrient culture with barley receiving nitrogen in different forms indicate that growth with ammonium salts is favourably influenced by the addition of copper, manganese, or certain other elements (Arnon, 1937).

A corollary to the harmful effect of certain soils on plant life, which can be ameliorated by the use of copper salts, is provided by certain diseases of animals. In various parts of the world sheep and cattle grazed on particular areas are liable to specific wasting diseases known popularly as 'coasty disease', 'licking disease', etc. Investigations in Australia showed the ameliorating action of copper and cobalt when fed in appropriate small quantities to the animals (Marston & Donald, 1937; Riceman & Donald, 1938, 1939). The internal organs of affected sheep develop certain diseased conditions in the cure of which copper is known to be important (Marston, 1937). In Tasmania (Philp, Dumaresq & Wilson, 1940) top dressing pastures with 20 lb. copper sulphate and 2 lb. cobalt sulphate per acre maintained sheep in perfect health in areas recognized as 'coasty', and similar results had been obtained elsewhere (Becker, Neal & Shealy, 1931).

Falling disease in Western Australia has been found by various analyses to be associated with a very low copper status of the pasture herbage and of the animals (Bennetts, Beck, Harley & Evans, 1941), the administration of copper supplements bringing the health and production of dairy cows up to normal. Sjollema had obtained similar results for 'licking sickness' in Europe (1933, 1938), showing that the copper content of the blood, liver, spleen and hair were reduced, and that with the administration of copper the return of haemoglobin values to normal was preceded by improvement in appetite.* In connexion with this method of treatment it is interesting to note that copper, as well as iron and nickel, is said to be capable of causing taint in milk through the chemical reactions which are initiated (Barkworth, 1938).

IV. IODINE

The important part played by iodine in animal physiology has been fully established, but it has so far proved impossible to demonstrate that it is also necessary to plants. It is often argued that it is unlikely that an element so important to animals, and one so usually obtained through the medium of vegetable food should not also be essential for the proper development of plants. If it is, then the amount required must be so extremely small that up to the present it has not been practicable to arrange a completely iodine-free environment in which to grow plants. Soil, water, nutrient salts, air, are all potential sources of supply, and the technical difficulties of purification are great.

* Copper drenches have also proved effective in the treatment of 'teart' disease in stock (Ferguson, Lewis & Watson, 1940).

Many analyses of the iodine content of foodstuffs and soil indicate that, broadly speaking, crops absorb iodine from the soil when it is available. The greatest concentration of iodine within the plant appears to be in the green parts, the leaves containing more than the stems and the stems more than the roots (Heller, Jones & Pursell, 1935). Powers (1939) also suggested that iodine seems to promote chlorophyll development, and that the nodule organisms of legumes are beneficially affected. Balks (1936) found that where thyroid troubles were most prevalent the iodine content of the soil and vegetables was less than in areas free from goitre. The use of fertilizers containing iodine has repeatedly been shown to raise the iodine status of the crops. Conner (1931) claimed that 5 lb./acre of potassium iodide was sufficient to raise the iodine content of crops very materially, but Dietz (1938) named a higher rate as being needed by lettuce and tomatoes, also indicating a toxic action if the application were too great. McHargue, Young & Calfee (1935) in Kentucky have obtained similar results, and also claim that in maize containing a relatively large amount of iodine the element was present in organic combinations suitable for assimilation by livestock and man. The Kentucky Experiment Station reports from 1930 onwards should be consulted for various analytical results both on soil and crops.

Apart from the general recognition that too much iodine is poisonous to plants, relatively little is known about the action of the element on growth. The germination of oats, wheat, rye and barley was adversely affected in this order by large doses of potassium iodide, the iodate and periodate being less toxic at parallel concentrations (Scharrer & Schropp, 1931). Similar checking of germination of barley and mustard had already been shown (Brenchley, 1924), without any indication of stimulation of these crops with smaller applications of iodine compounds. Loew (1925) made the somewhat fantastic claim for crop stimulation with 10 g. potassium iodide per acre, the maximum effect being reached with 25–125 g. according to the soil. Better growth of tomatoes was obtained in soil containing 10 p.p.m. of iodine than 50 p.p.m., the latter apparently being the maximum amount, in the form of potassium iodide, tolerated by this species (McHargue, 1937).

The possibility that iodine might be beneficial to plant growth is repeatedly coming to the front, because a relatively heavy amount is present in the natural fertilizer Chilean nitrate. Though no direct action on the plant has been proved (Giesecke, Schmalfuß & Rathje, 1938; Brenchley, 1943), it is known that sufficient iodine is supplied by the Chilean nitrate to affect the iodine content of crops, and it may well be that this in itself justifies the use of this form of nitrogenous fertilizer, especially in districts where thyroid troubles are manifest.

V. MANGANESE

During the last ten years comparatively little work has been done on the physiological connexion of manganese with plant growth, though earlier information on the practical side has been extended and consolidated.

The ameliorating action of manganese for grey speck disease of oats has been repeatedly confirmed from various parts of the world (Albert, 1934; Leeper, 1940; Steenbjerg, 1935), the disease seldom being reported from acid soils or those with *p*H above 8. Leeper states that the growth of oats on a manganese-deficient soil was greatly improved by raising the *p*H above 8·5 with sodium hydroxide. Experiments with basic slags on various types of manganese-deficient soils (Gericke, 1940) showed that the manganese in the slag was almost as effective as manganese sulphate in preventing grey speck disease. The yields of corn and kernel weight were improved, the seeds germinated better but yields of straw were not affected. This was confirmed by Rademacher (1940), who put the efficiency of the Mn in basic slag at about two-thirds of that in manganese sulphate. Gerretsen (1936, 1937) takes the view that grey speck is caused not only by lack of sufficient manganese, but by the presence or absence of certain bacteria. The typical specks on the leaves may be caused by ammoniacal products of decomposition which are produced by the bacteria in the roots of plants poor in manganese and pass upwards through the tissues. The micro-organisms, which are active between *p*H limits of 6·5–7·8, precipitate insoluble manganic oxides in the soil, thus causing deficiency of available manganese. This lack of manganese causes a decrease in photosynthesis, as the element plays an important part in the oxidation-reduction phenomena occurring during carbon dioxide assimilation. The lowered photosynthesis, in its turn, may account for diminished yields, reduced root systems and lowered resistance of the roots to invading micro-organisms, thus completing a vicious circle.

Peas affected with marsh-spot have a lower manganese content than healthy peas, and this was suggested by Lohnis (1936) as a possible cause of the disease. Heintze (1938) found that marsh spot is closely associated with soil reaction, less salt-soluble manganese being present in soils liable to the disease. The use of manganese sulphate, which controls the disease under field conditions, appreciably increases the salt-soluble manganese in the soils. The association of marsh spot and manganese was confirmed by de Bruyn (1939), who also stated that seeds in late developed pods of an individual plant showed more severe symptoms than seed from the first formed pods. Koopman (1937) and Lewis (1939) tried spraying with manganese salts in solution, and obtained an appreciable reduction in the incidence of the disease, spraying being most effective if carried out during the flowering period.

This method of spraying has been tested and proved efficient in various other cases of leaf chlorosis. Sugar beet yellows, caused by manganese deficiency (van Schreven, 1936*b*), can be controlled, and the crop yield increased by spraying (de Haan, 1937). With chlorotic beans on peat soil spraying with manganese sulphate may be more economical than treating the soil (Townsend & Wedgworth, 1936). Fruit trees exhibiting mottle leaf have also been benefited by late spring spraying with 0·25–1 % manganese sulphate solutions (Dippenaar, 1940). Certain ornamental plants showing chlorotic disorders in Florida also responded to spray treatment (Dickey & Reuther, 1938), but caution is advised until the response of individual species has been determined, in case of damage to the foliage.

Applications of manganese as a cure or preventive of deficiency disease may affect the plant composition apart from increasing the manganese content. In citrus, sugar formation was shown to be accelerated throughout the growing season (Roy, 1937), the fruits being heavier and firmer and the rinds and juice more intensely coloured. In potatoes manganese deficiency may cause reduction in total sugars and starch and increase in tyrosine (Smith & Nash, 1939). Tomatoes respond in various ways to manganese. The yield of fruits was found to be doubled by the application of manganese dioxide in addition to the fertilizer, the action of the salt being that of an oxidizing agent (Iyer, Rajagopalan & Subrahmanyan, 1935). The cracking of the fruit, which is largely associated with unfavourable moisture conditions in the plant, was reduced by the application of potassium permanganate to the soil (Iverson, 1938), the action in this case being attributed largely to the manganese itself rather than to direct chemical oxidation. The ascorbic acid content of tomatoes rises with increase in the available manganese in the soil, at least to the point beyond which manganese becomes toxic (Hester, 1941).

In connexion with the known toxicity of excess manganese for many plants McCool's work (1935) is of interest. In soy beans and buckwheat showing damage due to manganese treatment of the soil the injury decreased if the light intensity was reduced by shading. The decrease in yield of soy bean, snap bean and tobacco treated with manganese sulphate was, however, not prevented by shading plants. Variations in the manganese content of leaves, stems and roots were found under differing conditions.

VI. MOLYBDENUM

Molybdenum was recognized in plants by Demarcay in 1900 and possibly by earlier workers, but from about 1930 onwards more attention has been given to the element and improved methods of determination have been developed (Dingwall, McKibbin & Beans, 1934; Stanfield, 1935). Molybdenum is toxic to plants above certain limits, but quantities too small to damage herbage seriously may have very harmful effects upon animals feeding on it. Before 1940 it was acknowledged that 'teart' disease, causing intense scouring in cattle and sheep, is due to the presence of molybdenum in the herbage (Ferguson *et al.* 1940). The quantity taken up varies, grasses absorbing about half as much as clovers, so that aftermath including a good deal of clover is liable to cause more trouble from 'teart' than the earlier growth.

Toxic quantities of molybdenum may give rise to interesting phenomena in plants other than the usual features of reduced growth, thickened roots and lower dry weight. Sheffield (1934) found that salts of molybdenum added to soil produce cytological abnormalities in solanaceous plants which simulate those due to aucuba mosaic or Hy III disease, and that in *Solanum nodiflorum* the erect habit of the plant may change to trailing. Extending this work to other members of the Solanaceae, Warington (1937) found they are more susceptible to molybdenum poisoning than is barley. Toxicity is accompanied by characteristic colour changes—golden yellow in tomato and *S. nodiflorum*, and reddish yellow in potato tubers, due to the formation of yellow globules of a tannin-molybdenum compound within the cells. In tissues containing anthocyanin, blue granular accumulations also occur, apparently being compounds of an anthocyanin-molybdenum nature.

The molybdenum content of leaves from various trees, in mg./1000 leaves, ranged from 0·89 mg. in horse chestnut to 0·010 in *Prunus cerasifera* in samples taken in May, the quantity remaining constant or increasing in the later part of the year (ter Meulen, & Ravenswaay, 1935). It was also reported (Bertrand, 1939, 1940) that crucifers and legumes were particularly rich in molybdenum, ranging up to 4·5 mg. Mo/kg. of dry matter, the strawberry fruit contained 3 mg./kg., while the plumule and radicle of the bean had 53 mg. Mo against 2 mg. in the combined cotyledons and integuments.

Stimulation of growth of *Azotobacter* in nitrogen-free media by traces of molybdenum was detected in 1930 (Bortels, 1930), this observation being ratified by Bortels and other workers in later work which included various micro-organisms and the nitrogen-fixing blue-green algae of the Nostocaceae (Bortels, 1936, 1940). Nitrogen fixation was increased as much as 100-fold by molybdenum, the optimal concentration being about 1 : 50,000,000. In soil both molybdenum and vanadium compounds were found to stimulate the growth of *Azotobacter* and to increase the nitrogen content (Bortels, 1937). This element also had a direct bearing upon the nitrogen fixation and growth of leguminous plants, which are increased by treatment with sodium molybdate at the rate of about 1 g./sq.m., the beneficial effect on lucerne apparently being enhanced by the use of boron in addition (Bortels, 1941). This was not supported by Jensen (1941) who stated that nitrogen fixation in lucerne and clover was not influenced by molyb-

denum. Bortels (1938) also put forward the suggestion that high barometric pressure influenced the action of molybdenum and other rare elements on nitrogen fixation by bacteria. In view of these results it is interesting to note that Burk & Horner (1935, 1936) suggested that it might be needful to re-examine past work on *Azotobacter* in the light of possible molybdenum deficiency or contamination in the various experiments. According to Katznelson (1940) *Azotobacter* survived in certain soils when molybdenum and calcium carbonate were added alone or together, different soils varying in their ability to support the organisms.

Traces of molybdenum have been proved necessary for the proper development of the fungus *Aspergillus niger* (Steinberg, 1936, 1937, 1939) and a similar need exists in *Lemna minor* (Steinberg, 1941). With the higher plants there is the usual evidence of the toxic nature of molybdenum salts if present in too great supply, maize (Scharrer & Schropp, 1934), tomatoes, barley and buckwheat (Rose & Eisenmenger, 1937) reacting in this way. Parallel with this is the possible stimulation of the same species with traces of the element.

Bortels' claim that the efficiency of molybdenum is increased in the presence of boron was supported by Dmitriev (1938, 1939), who obtained increased yields of red clover seed by the application of 5 mg. Mo to 1 kg. soil in association with boron and other fertilizers. In some cases, as with peas, stimulation was obtained in nutrient cultures, but not in soil or sand (Bobko & Savvina, 1940). This increase was apparently associated with nodule development, none being produced in the absence of molybdenum.

The value of molybdenum for lettuce and asparagus was suggested by Arnon (1938), but as the molybdenum was added in a group of seven elements the beneficial action could not be fixed on any one of these. Later certain deficiency symptoms in tomatoes, shown by mottling and necrosis, were remedied by 1 : 100,000,000 molybdenum supplied as molybdic acid, no other element proving effective (Arnon & Stout, 1939). Spraying also appeared to be an efficient method of application, the molybdenum being absorbed through the leaves. Piper (1940), working with oats, found that molybdenum made no difference to the growth for the first five months, but that necrosis appeared on the leaves of the plants without molybdenum just at the time the panicles were about to emerge. The panicles all developed normally, but the grain only filled out when molybdenum was provided, remaining as empty husks in its absence. Hoagland (1941) indicated that Myrobalan plum seedlings showed characteristic deficiency symptoms if grown in nutrient solutions without added molybdenum. Much of this work must be regarded as preliminary, but there is sufficient evidence to suggest that in some conditions and with certain plants molybdenum may play an essential part. Rothamsted experiments with lettuces in nutrient solutions suggest an improvement with molybdenum at the rate of 1 : 10,000,000, the plants being heavier, deeper green and more healthy, apparently showing increased resistance to disease (Brenchley, 1943). The conditions which cause this benefit are, however, not yet definitely known, as it does not always occur and may be bound up with seasonal or other environmental factors. There is as yet no justification for the assumption that molybdenum is essential to the growth of all plants under all conditions, and much more work on the element remains to be done. If the suggestion that molybdenum increases the resistance of the plant to disease should be confirmed, it would be a matter of considerable economic importance in reducing the wastage among valuable crops.

VII. SELENIUM

Until recent years selenium received little attention from plant physiologists, though Awschalom (1921) and Levine (1925) indicated toxicity to germination and growth. Both workers used what are now recognized as high concentrations, of the order of 1 part in 10,000, but Levine observed growth increase with selenium compounds in greater dilution. Nemec & Kas (1920, 1921) found that the germination and growth of spores of various species of *Penicillium* were favoured by very weak sodium selenate, while Stoklasa (1922) put forward the idea that the poisonous action of selenium was neutralized by radioactivity in the nutrient medium. Several years later Hurd-Karrer (1933–8) revived interest in the element in a masterly series of papers dealing with the correlation between selenium and sulphur. The absorption of selenium and its toxicity to plant growth are directly affected by the amount of sulphur available. Selenites and selenates respond differently to sulphur treatment, as high concentrations of sulphur reduce the toxicity of selenates but increase that of selenites, though at low concentrations of sulphur selenite toxicity is reduced. On seleniferous soils the application of sulphates, gypsum or elemental sulphur reduces selenium absorption, and minimizes the risk, not only of plant poisoning, but of harmful effects to stock consuming the crops. Selenium is far more toxic to animals than to plants, and this can be taken advantage of to combat such pests as cotton stainer and pink bollworm by relatively low applications of selenites to the soil (Mason & Phillis, 1937, 1938).

Various workers (Beath, 1936; Beath, Draize & Gilbert, 1934; Beath, Eppson & Gilbert, 1937; Hoskins, 1938; Miller & Byers, 1937; Martin, 1936; Martin & Trelease, 1938) have investigated the distribution of selenium in plants and its toxicity to various crops, with occasional suggestions of beneficial action. Very light dressings of selenium encouraged the early germination of wheat (Perkins & King, 1938), while Trelease & Trelease (1938–9) suggested that the element improves the growth and is possibly essential for such plants as *Astragalus* spp.

which grow freely on seleniferous soils and which are regarded as indicators of the presence of selenium in soil. Compared with standards for other essential elements the optimal quantities are distinctly high, ranging up to 27 parts per million of selenite, at which point root growth is checked. Stanford & Olsen (1939) observed stimulation of growth in various cereals with very low concentrations of selenium, 1 or 2 parts per 1,000,000, the effect on grain production not being indicated.

From the scanty information available it is not yet possible to rank selenium among those elements that are essential or even beneficial to plant growth, though there are indications that circumstances may occur in which the element exercises these functions.

VIII. ZINC

Fundamental work on the effect of traces of zinc on the metabolism of the plant has rather fallen into the background for several years, but much has been done on the benefit derived from zinc treatment for such diseases as white-bud, mottle-leaf, and little-leaf or rosette. Chlorosis or white-bud in maize and other plants has been successfully controlled by sowing 10–20 lb. zinc sulphate per acre in the row before planting (Barnette *et al.* 1934–7). On some soils in Australia sown with wheat similar treatment increased the yield and resistance to disease (Forster & Hore, 1939; Milligan, 1940).

Rosette disease in pecan trees is accepted as being due to zinc deficiency, but other factors may co-operate in producing the result. Conditions of growth and exposure to light and heat may effect the development of the disease (Finch, 1936). The translocation of zinc was found to occur in the xylem, moving most rapidly in an acropetal direction. On tomato roots zinc deficiency may cause swellings and irregular distribution of root hairs (Eltinge & Reed, 1940), with other abnormalities in internal structure. Tannin, calcium oxalate and fats were found to be present in abnormally large amounts, and starch was absent from the vacuoles of affected cells.

Little-leaf or rosette disease of fruit trees was associated by Ark (1937) with the soil micro-flora as well as with zinc deficiency. In an affected orchard this was predominantly bacterial, while in a healthy orchard fungi were much more in evidence. McWhorter (1934, 1936, 1938) treated sweet cherry trees for little-leaf by boring holes in the trunk just below ground level and filling with powdered zinc sulphate, or alternatively by driving in zinc tacks ½ in. apart spirally round the trees. He was, however, not convinced that zinc deficiency is the sole cause of little-leaf, owing to the occasional unexplained recovery of affected trees. Hoagland, Chandler & Stout (1936) and Chandler (1937) support the idea that in some cases, if not all, soil micro-organisms may intervene to bring about the zinc deficiency in the plant. From a zinc survey of the soils of California, Hibbard (1940) found that though an adequate amount of zinc might be present, on alkaline soils it appeared to be unavailable to some species of plants. Also considerable accumulations of zinc may occur in the top soil under trees or other persistent vegetation.

The beneficial effects of zinc sprays on mottle-leaf of citrus have been repeatedly indicated (Parker, 1938; Powell & Matthews, 1936; Grimmett, 1938). In some cases the use of zinc sulphate sprays is said to increase the acid and juice content of the fruit, even if the application is made after the fruit is set (Camp & Reuther, 1935). Haas (1936) found that the use of zinc reduced the sucrose content of the leaves and roots of citrus and that the coating of leaves with zinc temporarily retarded growth. The efficiency of zinc treatments varies according to the material used, its concentration, methods of application and the way growth responds to treatment (Reed & Parker, 1937). A detailed study of the cytology of leaves affected with little-leaf was made by Reed (1938), indicating that the disease appears to promote cell growth rather than multiplication in the palisade parenchyma. He puts forward the speculation that zinc salts may catalyse oxidation processes in the cells and that in their absence the biochemical reactions may run the other way. In later work (1941) Reed carried on the investigation on cells of vegetative buds, finding an association between zinc deficiency and the early accumulation of phenolic compounds, which induce abnormal cell growth and development.

The zinc content of plants affected by deficiency appears to be on a lower level than in healthy plants (Grimmett, 1937). Treatment with zinc, either in the soil or by spraying, increases the zinc content of the leaves (Gaddum, Camp & Reuther, 1937). Analyses of weeds and wild grasses indicate that these can absorb more zinc than planted crops and so leave behind them sufficient available zinc to prevent the development of white-bud trouble in the succeeding crops of maize (Rogers, Gall & Barnette, 1939). At an earlier date Bertrand & Andreïcheva (1934) had found that for a variety of crops the green leaves were richer in zinc than blanched leaves on the same plant, a fact which may be significant in relation to the curing of chlorosis by zinc treatment.

Apart from the question of disease the addition of zinc salts to the ordinary fertilizer has been found to hasten the maturity of peas and swedes, but without increasing the yield (Teakle, Morgan & Turton, 1941).

IX. OTHER ELEMENTS

Arsenic. The extensive use of arsenicals as sprays and for insect control has focused attention upon its toxic properties and the possibility that crops might be rendered harmful for feeding purposes, a viewpoint which lies outside the scope of this review. Accumulation of lead arsenate sprays used for fruit

trees tends to render soil unproductive, hindering the full development of other crops sown on the areas (Vandecaveye, Horner & Keaton, 1936), but heavy applications of ferrous or ferric sulphate were effective in reducing the concentration of soluble arsenic, thus resulting in greatly improved growth of barley and alfalfa (Vandecaveye, Keaton & Kardos, 1938). High levels of mineral nutrients, either in soil or in water cultures, did not reduce the toxicity of arsenic (Crafts, 1939), but a claim was made that heavy leaching reduces the concentration of available arsenic in the soil (Crafts & Rosenfels, 1939). Calcium arsenate, when used for insect control, may not cause trouble from arsenic accumulation till 400 lb./acre is applied (Dorman, Tucker & Coleman, 1939), while light applications were even found to be beneficial to the growth of cotton on sandy loam (Dorman & Coleman, 1939).

Barium. In Dutch experiments barium chloride significantly increased the percentage of sugar recoverable from sugar beet, though the crop yield was not significantly affected (Bruinsma, 1940). With *Aspergillus* receiving optimum quantities of sulphur, small quantities of barium chloride increased the yield and acidity, larger amounts progressively decreasing both (Steinberg, 1936). Claims have also been made that traces of barium are stimulating to maize. Barium is said to be more toxic than stronium on the whole, the cereals increasing in sensitivity in the order wheat, oats, barley, rye (Scharrer & Schropp, 1937).

Cadmium. Though cadmium is toxic at very low concentrations, it may have some influence on the seed. Applications of cadmium chloride together with borax increased the yield of seed and the size of plants of red clover (Dmitriev, 1939). Dilute solutions of cadmium nitrate tended to stimulate germination of dormant timothy seed, but there was distinct toxicity of the metal ion even at 0·001 *M* concentration (Toole, 1939). With *Aspergillus niger* the presence of cadmium in the nutrient solution increases the copper requirement of the fungus (Mulder, 1939). In *Spirogyra* cadmium tended to increase protoplasmic elasticity in common with other chemically related elements (Northen & Northen, 1939).

Chromium. Suggestions of stimulation of various crops with high dilutions of chromium salts were obtained by Scharrer & Schropp (1935), though the border line of toxicity was easily reached. Attempts to substitute chromium for iron in water cultures with maize were unsuccessful. 'Yellow branch' of citrus, a serious disease in the Transvaal, occurs where the soil contains a considerable amount of chromium (Van der Merwe & Anderssen, 1937). In tea bushes affected by 'Witches Broom', Keiller (1939) found that affected leaves contained more chromium than healthy ones, the quantity increasing with the severity of the disease.

Cobalt. During the last few years work on the physiological effect of cobalt on plant growth has been completely overshadowed by the discovery that various wasting diseases of stock in different parts of the world are due to a deficiency of cobalt in the herbage (Askew, 1936–7; Askew & Maunsell, 1937), usually arising from a low cobalt status of the soils in the affected areas (Harvey, 1937; Underwood & Harvey, 1938). The diseases can be controlled by regular drenching of the animals with solutions of cobalt salts (Askew & Dixon, 1936), or, better, by the daily administration of about 2 mg. cobalt with the food. This can also be supplied by the provision of cobalt salt licks (Dixon, 1937) or limonite (McNaught, 1937) where the cattle have easy access to them. By the use of fertilizers supplying cobalt, such as specially prepared cobaltized superphosphate (Askew, 1939), the cobalt content of the herbage can be raised to a point which prevents the onset of wasting disease in the stock feeding upon it (Kidson & Maunsell, 1939). Analyses indicate that the cobalt content of pasture plants decreases at seasons when growth is retarded and increases in spring and summer when growth is at a maximum (McNaught & Paul, 1939; Rigg, 1938–9). It is impossible to refer to more than a few of the many papers on the subject, most of them coming from Australia, others from such widely separated areas as Canada (Bowstead & Sackville, 1939) and Scotland (Corner & Smith, 1938). A paper dealing with the relative poisonous effects of cobalt, nickel and copper on barley and broad beans gives many of the earlier references up to 1938 (Brenchley, 1938).

Lead. The possible stimulation of growth by lead compounds has frequently been suggested. Keaton (1937) found that near Washington very heavy dressings of lead carbonate had no harmful effect on barley, and that usually plant growth was moderately stimulated by lead compounds. Scharrer & Schropp (1936) also obtained stimulation of maize, barley and oats, the effect on wheat being uncertain, giving the curious result that small amounts seemed injurious while large amounts were beneficial. Cocorullo (1938) claimed that the germination of seeds of radishes was encouraged by growing them in lead pots. Hooper (1937) found that the presence of lead in the soil does not seriously affect plants, and that the barrenness of lead mine dumps probably results from the wind-borne lead dust which blocks the stomates of the plants. The action of lead compounds on tree seedlings varies with the species (Wieler, 1938), but all are harmful when the soil is made alkaline by liming.

Lithium. Absorption of lithium chloride from dilute solutions by plant cells, as in *Chara ceratophylla*, is very rapid even in the dark, and is stimulated by light (Collander, 1939). In *Spirogyra* lithium and caesium both decreased protoplasmic elasticity, and from tests with other elements it was concluded that the effect of an ion in decreasing protoplasmic activity is due to its chemical activity rather than to its valency (Northen & Northen, 1939). The growth of yeast cells

may be slightly stimulated by lithium (Lasnitzki & Szorenyi, 1934).

Nickel. Little work on nickel has recently been carried out, except for water culture work which showed the individuality of plant response to poison by the great variation in growth in the borderline concentrations just below those which caused marked depression (Brenchley, 1938). At the time that the value of cobalt was recognized as a preventive of wasting diseases it was suggested that the addition of nickel salts made the drench still more effective (Dixon, 1937), but this does not seem to have been followed up.

Rubidium. Most of the recent work on rubidium has some bearing on its relation to potassium. Spectrographic examination showed the presence of rubidium in sugar beet in almost the same quantity as copper, zinc, and iron, the ratio Rb/K also being determined (Breckpot, 1936). The selectivity for rubidium varies considerably between different plant species, and the quantities of potassium, rubidium and caesium absorbed by plants were found to be correlated with each other (Collander, 1937). The toxic action of rubidium, and also of strontium, was mitigated by increasing amounts of potassium supplied to wheat and barley in water cultures (Hurd-Karrer, 1937). In certain of the lower fungi, as *Aspergillus niger* and *Saccharomyces*, and some bacteria, the only element capable of replacing potassium for nutritive purposes is rubidium, but the yield is smaller (Rahn, 1936). Similar replacement of potassium by rubidium has been observed in *Nitzschia closterium* (Stanbury, 1934). The most important recent work on the function of rubidium in plant growth is that of Richards (1941). At very low levels of potassium growth nearly ceases at the first or second leaf stage and many plants die, but the addition of rubidium to the nutrient solution enables growth to proceed almost normally. Too heavy a dose of rubidium is toxic, but over a wide range of concentrations the element increases total growth very considerably. Rubidium also seems to retard the rate of entry of phosphorus, especially in solutions high in calcium.

Strontium. Various crops, including cereals and peas, grown in sand and solution cultures have shown stimulation with traces of strontium, plants requiring the least strontium for stimulation being the most easily poisoned by larger amounts (Scharrer & Schropp, 1937*b*). In *Elodea* protoplasm calcium, strontium and barium can be firmly bound, and can replace each other (Mazia, 1938).

Thallium. The toxic effects produced by solutions of thallium salts on tobacco plants closely resemble the symptoms characteristic of 'frenching', including chlorosis (Spencer, 1937), a close correlation existing between resistance of species to the poisoning and their resistance to frenching. Although the same effect was obtained by van der Veen (1938) he did not consider it proved that frenching is due to thallium toxicity. Borzini (1935) found that seeds placed to germinate with dilute solutions of thallium salts absorb thallium ions through the roots; these pass into the stems and cotyledons, reduce the rate of germination and growth, and inhibit the formation of chlorophyll in a number of species, including cereals and leguminous plants.

Vanadium. Vanadium in larger amounts than occur normally in basic slag has been found to stimulate the growth of red clover (Gericke & v. Rennenkampff, 1940). In the slag itself the vanadium is less active, owing to the calcium oxide present, and the vanadium content of slag is considered to be a wholly favourable factor, without any danger of toxicity from the amount present.

X. SUMMARY

During recent years more work has been done on the practical value of minor elements than on their physiological function in the economy of the plant. There are still very few elements of which there is definite proof that traces are essential to growth.

The importance of *boron* in plant nutrition is firmly established. Large numbers of species are now known to suffer if the boron supply is insufficient and much commercial loss can be avoided by the judicious admixture of boron compounds with fertilizers. The value of Chilean nitrate is enhanced by its boron content. The part played by boron in the metabolism of the plant, however, still remains obscure.

With *copper* attention has been focused on the value of copper salts in rendering fertile peat, or other highly organic soils, where they are brought under the plough. 'Reclamation disease' can be prevented by small dressings of copper sulphate before sowing. There is little direct evidence that copper is actually essential to the plant, though the assumption is generally made from the weight of indirect proof.

Plants may play an important part as carriers of the *iodine* that is so necessary for animals and human beings, but owing to technical difficulties it has not been possible to establish whether or not iodine is equally necessary to the plants themselves.

The value of *manganese* as an ameliorating agent for certain plant diseases has become increasingly evident, grey speck of cereals, marsh spot of peas and certain chlorotic disorders yielding to applications of manganese salts to the soil or as sprays on the foliage. The plant composition may be affected by treatment. Toxicity due to excess manganese may be controlled to some extent by variations in light intensity.

Molybdenum in herbage is recognized as an important causal factor in some animal disorders as 'teart' disease. In plants excess produces characteristic morphological and cytological conditions, but there are indications that in some circumstances traces of the element may prove beneficial.

Selenium toxicity in plants can be controlled by the judicious use of sulphur on seleniferous soils. As the absorption of selenium is reduced in this way, the risk of poisoning for animals feeding on the herbage is also minimized. Little definite evidence of benefit to growth due to traces of the element is yet available.

Among other elements comparatively little is known as to whether any one or more may be essential for plant life.

Isolated instances, particularly with fungi, indicate that such possibilities exist, but much work remains to be done before any assumption in this connexion can be made. The weight of evidence, indirect if not direct, with boron, copper, manganese, and zinc goes to indicate that each of these may play a vital role in plant development, though the exact conditions required and the extent to which each element is essential still remain subjects for investigation.

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OSMOTIC REGULATION AND THE FAUNAS OF INLAND WATERS

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I. INTRODUCTION

From geological and anatomical evidence it is quite probable that the ancestors of the major phyla of animals originated in the sea. Physiological evidence is also available. Sea water possesses just that combination of stable chemical and physical properties which make it the most physiologically favourable of all natural inorganic environments (Henderson, 1913). The small changes in the composition of sea water which may have occurred since the beginning of organic evolution are not sufficient to affect this conclusion. The main courses of evolution have been marked by progressive emancipation from the limitations imposed by the environment: protective mechanisms have been developed giving greater and greater independence, while physiologically difficult environments have been successfully colonized. There are many aspects to this problem of emancipation, but we are here concerned in tracing the probable steps by which marine animals got that control over the concentration of their internal medium which enabled them to invade fresh waters. We shall also consider the invasion of inland saline waters, an evolutionary side-line which has received little attention.

Many Protozoa and a few coelenterates, which have no body fluids, have managed to invade fresh waters by virtue of some intracellular regulating mechanism about which we know little, although the contractile vacuole of Protozoa has certainly played an important part (Kitching, 1938*), and there is no doubt, as we shall see later, that adaptation of the tissue cells to a changed body fluid has been one factor in the invasion of fresh water by Metazoa with body fluids. It is of course true that with living organisms there is no such thing as a static equilibrium, and that even the most primitive of marine animals maintains a dynamic steady state by which a difference in concentration of several ions is established across the external membranes and which can be maintained only by some active process (ionic regulation). But osmotic regulation, or the regulation of the total concentration of the body fluids, was a later development without which leaving the sea would have been impossible.

The osmotic pressure of a solution is exerted only when it is separated from the solute by a membrane of well-defined semi-permeable properties. The membranes separating the body fluids of animals from the external medium do not have these definite properties and probably do not remain stable. The use of the word 'osmotic' is therefore unfortunate, but is too far ingrained by usage to be discarded. In using the term 'osmotic regulation' we shall always mean the regulation of the total concentration of the body fluid without implying the nature of the forces against which the mechanism has to contend. Together with the mechanism of secretion it is an aspect of that fundamental biophysical problem of the movement of substances in solution against a concentration gradient.

Research on osmotic regulation (see reviews by Schlieper, 1930,* 1935;* and Krogh, 1939) has been chiefly concerned with finding the relation between external and internal concentrations, locating the organs responsible, and correlating the effectiveness of the mechanism with the actual needs of the animal in its normal environment. In a hypotonic medium (dilute sea water or fresh water) the internal concentration may be controlled through excretion of water by special organs or by the uptake of salts through the gut or body surface. These two processes may be connected since the vertebrate kidney (Richards, 1938), and perhaps all excretory organs producing a hypotonic urine, do so by the active reabsorption of salts from a body-fluid filtrate (Schlieper & Herrmann, 1930; Peters, 1935). In a hypertonic medium (e.g. inland saline waters) the reverse must occur, though the active excretion of salt has been definitely demonstrated only in the special case of marine teleost fishes, whose blood is hypotonic to sea water (Smith, 1932; Keys, 1933). This review is not concerned with the details of these mechanisms, but the course of the evolution of osmotic independence will be deduced from the comparative study of body-fluid concentration in relation to that of the environment, and an attempt will be made to assess the contributions made by the various regulatory mechanisms to the achievement of this independence. This is admittedly as speculative a proceeding as the deduction of the course of structural evolution from the comparative anatomy of living animals, and the data available are even less complete. But it is hoped that in assessing the possible evolutionary significance of the facts we may stimulate further research, though the hypotheses advanced may later require considerable modification.

II. NON-REGULATING BRACKISH WATER ANIMALS

Invasion of brackish water by marine animals has occurred in four main types of habitat: (i) coastal lagoons and salt marshes (Nichol, 1935; Howes,

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1939), (ii) the mouths of streams of steep gradient where the salinity fluctuations are rapid and confined to the intertidal zone (Pantin, 1931), (iii) estuaries where the salinity changes due to tidal movements may extend over many miles and are complicated by layering (Alexander, Southgate & Bassindale, 1932; Goodhart, 1941), (iv) the special habitat formed by the Baltic Sea where there is a permanent gradient of salinity, conditions being more or less permanent in a given area (Välikangas, 1933). The above selected references to ecological work on these habitats give data on the extent to which different species have penetrated into dilute sea water; from them further references can be obtained. Of particular interest are the data of Välikangas on the Baltic. Many typically marine animals live there in considerably diluted sea water, e.g. *Aurelia aurita* in 6 °/₀₀, *Membranipora pilosa* in 4 °/₀₀, *Mytilus edulis* in 4·5 °/₀₀, and *Mya arenaria* in 5 °/₀₀ salinity, oceanic sea water being 35 °/₀₀. Experiments have shown that the osmotic pressure of the jelly of several marine medusae, including *Aurelia aurita*, follows closely that of sea water when diluted (Fredericq, 1901; Macallum, 1903; Botazzi, 1908; Bateman, 1932), and the same is true of the blood of *Mytilus edulis* (Conklin & Krogh, 1938). There is in fact a large number of marine animals which, though lacking the mechanism for osmotic regulation essential for penetration of fresh water, can yet live in much diluted sea water. How they do so is obscure. In experiments the muscles and cilia of marine individuals of *Arenicola marina* and *Mytilus edulis* fail to function at salinities in which these species live in the Baltic (Wells & Ledingham, 1940; Wells, Ledingham & Gregory, 1940). There may be brackish water varities; or the tissues from marine individuals might work at these low salinities if acclimatized exceedingly slowly.

The work of Wells & Ledingham (1940) on polychaete worms has thrown some light upon this kind of adaptation. They studied the effects of diluting the medium upon the spontaneous contraction of isolated muscle preparations. The muscle of the mainly marine *Perinereis cultrifera* is more sensitive to dilution than that of the estuarine *Arenicola marina*, both of which fail to regulate to any significant degree the concentration of their body fluids. The muscle of *A. marina* is in turn more sensitive than that of *Nereis diversicolor*, which is naturally found in waters of lower salinity and whose body fluid, though regulated to some extent in dilute sea water, suffers a considerable dilution (Fig. 1). But with a gradual (exponential) dilution of the medium it became clear that, within the limits of the salinity tolerance of the muscle, it is the *rate* of dilution which is the determining factor. Reference to the body-fluid dilution curves of *Arenicola marina* and *Nereis diversicolor* transferred direct to 25 % sea water (Beadle, 1937) showed that protection of the intact animals from the effects of sudden dilution of the sea water is got by a 'damping' of the body-fluid dilution curve to the extent required for normal muscle functioning (Wells & Ledingham, 1940).

Of the polychaetes investigated *N. diversicolor* alone possesses the rudiments of an osmoregulatory mechanism (Fig. 1). But the experiments of Wells & Ledingham (1940) show that this worm could probably survive, without such a mechanism, in the lowest salinities in which it is normally found. No doubt there are many brackish water animals which are more than adequately protected against the salinity fluctuations which they encounter in nature. A similar phenomenon is found with several fresh and inland saline-water species (see below). Either extreme situations arise in nature more often than we suppose which tax the resistance of these animals strongly enough for selection to operate, or some of the factors which make for resistance may be of a non-adaptive character. Alternatively, the salinity tolerance range of a species determined in the laboratory is perhaps not a true measure of its ability to become permanently established under natural conditions. Salinities outside the natural but within the experimental range may perhaps render it less resistant to other factors in the environment.

III. OSMOREGULATION IN BRACKISH WATER ANIMALS

Fig. 1 shows that there are great differences in the body-fluid concentration levels maintained by brackish water animals, ranging from the Australian crab *Heloecius cordiformis*, which is practically homoiosmotic, to the polychaete worm *Nereis diversicolor*, which by comparison maintains a relatively small degree of hypertonicity.* It is curious that the crabs *Heloecius*, *Eriocheir* and *Carcinus* should maintain the concentration of their body fluids at such a high level when other animals, such as *Nereis diversicolor*, can live with comparatively little regulation. It seems that there are two possible methods of adaptation to dilute sea water, (i) by regulating the body fluid and thus insulating the tissues from the salinity changes of the environment, and (ii) by adapting the tissues to a diluted body fluid. These two methods appear to have been adopted in different proportions by the animals represented in Fig. 1.

In some cases (e.g. *Cancer pagurus* Duval, 1925

* *Gammarus locusta* gives a curve similar to that of *Eriocheir*, but is not readily adapted to salinities lower than 130 mM. (Beadle & Cragg, 1940*a*). The course of the curve of the prawn *Leander squilla* is close to that of *L. serratus* (Panikkar, 1941). Assuming a relation between Cl′ and total blood concentration similar to that for *Gammarus duebeni* (Beadle & Cragg, 1940*a*) the curve deduced from the Cl′ figures of Bogucki (1932) for the brackish water variety of *Chiridotea entomon* would follow a course almost identical with that for *Gammarus duebeni*, which it also resembles in not being adapted to fresh water.

and *Perinereis cultrifera* Wells & Ledingham, 1940) the degree of hypertonicity of the blood in dilute sea water is so slight as to have no obvious functional significance. But in one respect the osmoregulatory mechanism differs from that of fresh-water animals: those which have been investigated produce a urine which is isotonic with the blood in all dilutions of sea water to which they can be adapted (Fig. 1). The excretory organs are therefore not concerned in the mechanism, and in fact their action results in a loss of salts. In order to maintain the concentration of the body fluid there must consequently be some active uptake of salts from an external source. But the question arises as to whether the animals rely entirely on absorption from the food, or whether salts are also taken up from the environment against a concentration gradient through some or all of the body surfaces. Nagel (1934) showed that if *Carcinus*, acclimatized to dilute sea water, is transferred to water more concentrated but still just hypotonic to the blood, the concentration of the latter is raised. Salts are therefore actively absorbed from the medium under these conditions. (The active uptake of individual ions was demonstrated by Webb (1940).) Since *Carcinus* does not significantly change in weight when the medium is diluted (Bethe, 1934) it is evident that the rise in blood concentration is not due to expulsion of water. By the same method active absorption was demonstrated in *Nereis diversicolor* (Beadle, 1937), *Palaemonetes varians* and *Leander serratus* (Panikkar, 1941), and *Gammarus duebeni* (Beadle & Cragg, unpublished). In other words the dilution experiments illustrated by the curves in Fig. 1 are reversible, and addition of sea water to the diluted medium is answered by a rise in blood concentration, and from the evidence we must conclude that active absorption of salts from the medium is an important component of the osmoregulatory mechanism in brackish water animals. But it is also likely that under natural conditions the food must supply a proportion of the blood salts.

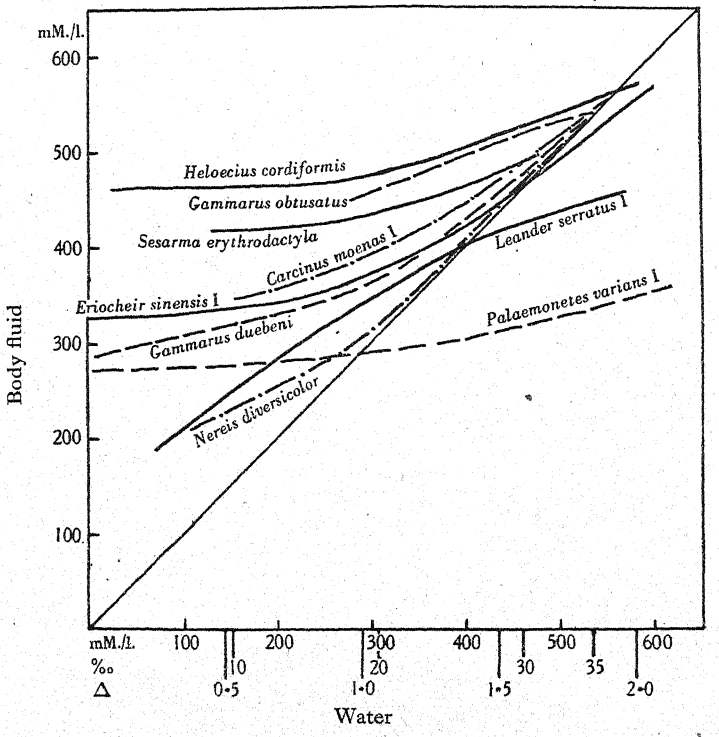

Fig. 1. Relation of the external to the internal medium in various brackish water animals (*Heloecius cordiformis*, *Sesarma erythrodactyla* (Edmonds, 1935); *Gammarus obtusatus*, *G. duebeni* (Beadle & Cragg, 1940*a*); *Carcinus moenas* (Dural, 1925; Picken, 1936); *Eriocheir sinensis* (Scholles, 1933); *Leander serratus*, *Palaemonetes varians* (Panikkar, 1941*a*); *Nereis diversicolor* (Beadle, 1937)). The left-hand ends of the curves show, on the abscissa, the approximate low-salinity tolerance limits. I, urine isotonic with body fluid.

There is some evidence to show that brackish water animals are more capable of retaining salts against a concentration gradient than marine animals, and this would of course relieve the strain put upon the osmoregulatory mechanism. This is in practice difficult to distinguish from active uptake, but Nagel (1934) showed that sodium iodide added to the medium penetrates into the blood and is subsequently lost in pure sea water more slowly in the case of *Carcinus* than of the purely marine *Hyas arenaria* and *Portunus puber*. It is not however easy

to reconcile this apparent decrease in permeability to salts with the development of a mechanism for active absorption. The problem will be more profitably discussed in connexion with fresh-water animals.

The high degree of hypotonicity maintained in sea water by *Leander serratus*, *L. squilla* and *Palaemonetes varians* (Fig. 1) is of considerable interest. It has been discussed in detail by Panikkar (1941), who suggests that 'they have been derived from prawns that have been established in fresh water and developed a high degree of osmotic regulation, but for some reason they began to penetrate back into brackish water and then into the sea'. It is of course a mechanism for hypertonic regulation that is required in fresh water, and what is presumably meant is that their visits to fresh water in the past have induced them to regulate the concentration of their blood at a relatively constant and intermediate level in face of salinity changes in both directions. There is not much more that can be said for or against this theory, but it is worth noting that animals living in excessively saline waters, whose powers of hypotonic regulation are particularly well developed, are mainly of fresh-water origin. Moreover, the euryhaline teleosts (eel and salmon) have a two-way regulating mechanism, and they and the marine teleosts, all of which maintain hypotonic blood, may perhaps be derived from fresh-water ancestors secondarily returned to the sea (Smith, 1932). Hypotonic regulation is here effected by absorption of sea water from the gut and excretion of chloride through the gills (Smith, 1932; Keys, 1933). Nothing is known of the mechanism in the above prawns, but, unlike the marine teleosts, they do not suggest a fresh-water ancestry by producing a urine hypotonic to the blood. On the other hand, there are several marine decapod Crustacea which are slightly hypotonic in sea water (tabulated with references by Panikkar, 1941). It is again difficult to avoid the suspicion that the capacity for hypotonic, like hypertonic, regulation may be non-adaptive in some marine animals.

IV. PENETRATION OF FRESH WATERS

It is a striking fact that of all the brackish water animals referred to in Fig. 1, only the crab *Eriocheir* has taken the final plunge into fresh water. The osmoregulatory mechanism in *Heloecius* is apparently more developed, yet it cannot survive unless the medium contains slightly more salts than normal fresh water (Edmonds, 1935). The brackish water crustacean *Gammarus duebeni*, too, can live in such low concentrations of sea water (*ca.* 1%) that a further dilution to fresh water might be expected to present no obstacle. Whatever the nature of the osmoregulatory mechanism, the strain put upon it is surely dependent upon the difference in concentration between internal and external media, and, if we assume that when *Heloecius* and *Gammarus duebeni* are in very dilute sea water it is strained to the limit, there seems no obvious reason why the blood concentration should not be lowered by a small amount to make the final step possible. It is of course true that the relative proportions of the ions in fresh waters are very different from those in the sea, and this might be expected to influence the regulatory mechanism either directly or by affecting the permeability of the body surface. But such an explanation will not hold for *G. duebeni*, which cannot live in sea water diluted below 1% but remains healthy in a pure sodium chloride solution isotonic with 1% sea water.

There are two lines of investigation which may ultimately throw some light upon this problem. Some brackish water species have given rise to varieties properly adapted to fresh water, several of which seem to be structurally identical with the original. Thus the isopod *Mesidothea* (*Chiridotea*) *entomon* of the Baltic Sea is found as a fresh-water variety in inland lakes of Sweden, Russia and North America (Ekman, 1919; Gurjanowa, 1930; Bogucki, 1932). The amphipod *Corophium curvispinum*, which is considered to have first invaded fresh waters from the Black Sea and Caspian Sea, is now widespread in the Volga basin, in Central Europe as far as northern Germany, and has recently been reported in fresh waters of England (Wunsch, 1920; Crawford, 1937*a*). Similarly, the mollusc *Hydrobia* (*Paludestrina*) *jenkinsi*, an inhabitant of brackish water over most of its range in northern Europe, has in recent times been penetrating fresh waters in various parts of Britain (Ellis, 1926; Boycott, 1936). The structural features which distinguish the marine and fresh-water varieties of the north European Crustacea *Gammaracanthus loricatus*, *Pontoporeia affinis* and *Pallasia quadrispinosa*, are mainly concerned with size relations, and the fresh-water variety *relicta* of the Arctic Ocean *Mysisoculata* is another well-known example (Ekman, 1919).

It is not known whether the fresh-water forms of the above species differ from the brackish in the nature of the osmoregulatory mechanism or whether the brackish water forms can in fact regulate their blood successfully in fresh water but are not adapted to some other factor in the fresh-water environment such as food supply. The difference may also lie in the process of reproduction, since a species cannot establish itself in a new environment without provision for the adaptation of the young stages. This may have occurred in the variety of *Palaemonetes varians* found in the fresh waters of some Mediterranean countries, whose eggs are larger and larval development reduced compared with the brackish variety occurring in most parts of Europe (Sollaud, 1923, 1932). As Needham (1930) has pointed out, the eggs of fresh-water animals must contain at the start sufficient mineral matter for the needs of the embryo, this is absorbed from the sea water during the development of some marine invertebrates. It

should be possible to decide whether the larger size of the fresh-water variety's egg is evidence of adaptation in this sense or whether in addition it enables the embryo to develop within the protective egg membrane until its osmoregulative mechanism is functional. This appears to be the case with the trout egg (Krogh & Ussing, 1937). But if it can be shown, as seems likely, that in at least some cases the necessary change has occurred in the regulatory mechanism of the adult whereby it is adapted to fresh water, then comparative experimental work on the two varieties should prove illuminating, since other variables are reduced to a minimum and there is even the possibility of applying the technique of genetical analysis.

These conditions are probably realized with *Gammarus duebeni*. It is a species widespread in the brackish waters of northern Europe. But in some streams of western Britain it is found in pure fresh water (Crawford, 1937*b*; Beadle & Cragg, 1940*b*). It inhabits the inland lochs of the island of Barra (Forrest, Waterston & Watson, 1936) and in Ireland it is the commonest species of *Gammarus* in the fresh-water loughs (Reid, 1939). Comparative experiments by Beadle & Cragg (1940*c*) on the two forms showed that they are indeed physiological varieties. After acclimatizing both to 2% sea water, the fresh-water variety retained enough chloride in glass-distilled water to survive for several days at least, whereas the brackish variety lost chloride rapidly and died. Adaptation to fresh water in this species appears to have been effected by the perfection of a mechanism for the retention of salts, independently of, and in addition to, that for active absorption from the medium which the brackish water variety possesses (Beadle & Cragg, unpublished). Since the technique for the genetical study of *Gammarus* is so well advanced (Sexton & Clark, 1936) its application to the problem of these varieties of *G. duebeni* is likely to produce interesting results. It is remarkable that, though in some coastal streams (e.g. at Kynance, Cornwall (Crawford, 1937)) it has penetrated the entire course, in the Hebridean islands of South Rona and Raasey it has reached a vertical height of only a few feet above spring-tide level, though the upper reaches are devoid of competition from other *Gammarus* species (Beadle & Cragg, 1940*b*). In both cases it extends also into the intertidal zone. Have we here a case of two genetically distinct but intergrading populations and, if so, to what extent is there interbreeding? It is not yet known whether there is any difference between the two varieties with respect to reproduction and life history. A combination of physiological, ecological and, where possible, genetic work on fresh-water varieties of brackish water species in general is likely to shed light on the evolution of fresh-water life.

A second line of attack is suggested by the possibility of acclimatizing marine and brackish water animals to salinities well below their normal range and even to fresh water by extremely slow dilution of the medium. It is a common experience that if a marine animal is transferred through a series of decreasing salinities its tolerance range is greater than when the transfer is direct. Topping & Fuller (1942) found that *Nereis virens* when put direct into fresh water lived only 6 hr., but when the medium was gradually diluted over 19 days they survived for as long as 61 hr. in fresh water. But several workers have claimed that, if the dilution is spread over many weeks or even months, some species can be permanently acclimatized to fresh water. Thus Beudant, in 1816, reported that in an experiment of $5\frac{1}{2}$ months the marine molluscs *Mytilus*, *Ostrea*, *Cardium* and *Patella* were induced to live in fresh water (see Schlieper, 1933). A similar result was obtained by Sexton (1928) with *Gammarus locusta* and *chevreuxi* by diluting the medium over a period of 'several weeks'. It is not clear from Sexton's paper whether the medium was ultimately free from traces of sea water nor to what extent the process entailed selection. But, since the reproductive cycles were apparently maintained throughout, selection may well have operated. But in the experiments of Beudant and of Schlieper (1929) it was a case of progressive adaptation of individuals. If these experiments are repeated, especially those on *Gammarus* spp., a comparative study of the adapted and original animals is likely to be fruitful.

If we now consider the blood concentrations of fresh-water animals (shown along the ordinate in Fig. 2), we find the variation as great as with brackish animals. *Eriocheir* is also included since the majority of its adult life is actually spent in fresh water. It seems that most fresh-water animals differ from those living in brackish water in that the excretory organs assist in osmoregulation by the production of a hypotonic urine. But it is remarkable that the two known exceptions (*Eriocheir* and *Telphusa*) are those which maintain the highest blood concentration. These may perhaps be regarded as physiologically brackish water animals which have penetrated fresh water without an essential change in the regulatory mechanism. From a comparison of the blood concentrations summarized in Fig. 2 it is tempting to suggest that during the evolutionary history of fresh-water animals the first regulatory mechanism to be developed was one which maintained a high blood concentration not much lower than sea water. In animals at this stage (e.g. *Telphusa*) the tissues are protected from a drastic dilution of the blood, but the mechanism is not supported by the excretory organs. The next step, we might suggest, was the adaptation of the tissues to a lower blood concentration which could be more easily maintained and the simultaneous development of renal reabsorption. This is admittedly speculative, but there are certain facts which support the hypothesis. In view of the probable selective advantage of the second step, it is not surprising that

the majority of existing fresh-water animals have reached it. We should also expect those brackish water animals which have almost or completely adapted themselves to fresh water (*Heloecius*, *Eriocheir*, *Gammarus duebeni* and *Palaemonetes varians*) to maintain high blood concentrations. Some suggestive evidence comes from the *Gammarus* species (Beadle & Cragg, 1940*a*, *c*). The blood concentration of the fresh-water variety of *G. duebeni* when in fresh water is the same as that of the brackish form in very dilute sea water, and both are nearly twice the concentration of the blood of *G. pulex* (Figs. 1, 2). More figures are required for the fresh and brackish water varieties of other species. Of interest also would be the blood concentrations of those fresh-water species whose nearest relatives are marine, such as the mollusc *Dreissenia polymorpha* widespread in Europe, the polychaete worms of the genus *Dybowscella* in Lake Baikal, and the serpulid *Marifugia cavatica* in the caves of Herzogovina (Hemplemann, 1934).

Direct evidence for the existence of a renal reabsorption mechanism is obtainable only from fairly large animals, but no animal with a higher blood concentration than 250 mM./l. has yet been found to produce a hypotonic urine. It has, however, been known for some time that the canal of the excretory organ (antennal gland) of several fresh-water Crustacea is longer than in nearly related marine and brackish water species (Rogenhofer, 1908; Schlieper & Herrmann, 1930). The inference is that a short canal is associated with isotonic and a long one with hypotonic urine production. *Telphusa fluviatilis* and *Eriocheir sinensis* are the expected exceptions; although inhabiting fresh water their urine is isotonic and the excretory canals are short like those of *Carcinus* and *Cancer* (Schlieper & Herrmann, 1930).

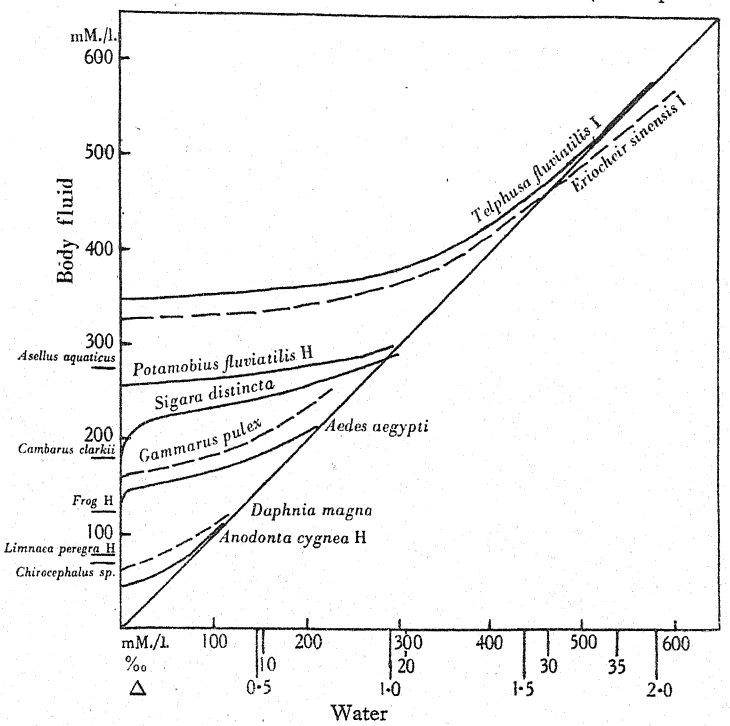

Fig. 2. Relation of the external to the internal medium in various fresh-water animals (*Telphusa fluviatilis* (Duval, 1925; Schlieper & Herrmann, 1930); *Eriocheir sinensis* (Scholles, 1933); *Potamobius fluviatilis* (Hertmann, 1931; Bujucki, 1934; Picken, 1936); *Sigara distincta* (Claus, 1937); *Gammarus pulex* (Beadle & Cragg, 1940*a*); *Aedes aegypti* (Beadle, 1939); *Daphnia magna* (Fritzsche, 1916); *Anodonta cygnea* (Duval, 1925; Picken, 1937); *Asellus aquaticus* (Widmann, 1935); *Cambarus clarkii* (Lienemann, 1938); frog (Macallum, 1903). *Chirocephalus* sp. (Panikkar,; 1941*b*)). The right-hand ends of the curves show the approximate high-salinity tolerance limits. I, urine isotonic with body fluid; H, urine hypotonic to body fluid.

That this canal is in fact the site of salt reabsorption from a previously isotonic blood filtrate was proved by Peters (1935) through microanalyses of chloride in the fluid from different parts of the gland. In their osmoregulatory action these organs therefore function in the same manner as the kidneys of fresh-water vertebrates (Richards, 1938). It is thus possible from the structure of the antennal glands of Crustacea, too small for direct estimation of the urine concentration, to predict the probable presence or absence of the reabsorption mechanism, whose existence in the fresh-water *Gammarus pulex* and absence from the marine *G. locusta* have been inferred in this manner (Schwabe, 1933). It would be

simple to extend these observations to the fresh-water variety of *G. duebeni* which we should *a priori* expect to lack the mechanism. The hypothesis we have been developing concerning the stages in the evolution of fresh-water animals has been based on data derived mainly from the Crustacea. It may be that further work will show some other groups to have lowered their blood concentration during the brackish water phase. *Nereis diversicolor* might well represent a stage in this kind of adaptation where the tissues are already adapted to a considerable lowering of body-fluid concentration.

In spite of the reabsorption mechanism, some salt will inevitably be lost from the excretory organs and by other routes in fresh-water animals, and some form of active absorption from an external source is essential. We have already concluded that active absorption from the medium is an important component of the osmoregulatory mechanism in brackish water animals. To what extent is this true of fresh-water animals? Krogh, who has been responsible for most of the research in this field, has reviewed the results in his recent book (Krogh, 1939). After dilution of the blood by keeping the animals in distilled water, it was shown that many fresh-water animals will absorb ions from concentrations of the order of natural fresh waters, and in some cases (e.g. *Potamobius fluviatilis* and *Rana esculenta*) chloride can be removed from the medium until there remains too little for detection. But most of the animals investigated cannot absorb chlorine ions from concentrations lower than 0·1 mM./l. There is no direct evidence that this mechanism is essential for the regulation of the blood under natural conditions, and fresh waters certainly exist which support a rich fauna but contain a concentration of chloride less than 0·1 mM./l. (Krogh, 1939, p. 204). The question can only be decided in individual cases by examination of the composition of the water in relation to the absorbing powers of the animal and to its feeding habits. It is possible that some species are dependent upon absorption from the medium at certain periods only, when food is not available. On the other hand, the phyllopods *Branchipus grubii* and *Lepidurus productus* are unable to absorb ions from fresh water and are presumably entirely dependent upon food (Krogh, 1939, p. 98). There is little evidence to show that, within the limits found among normal fresh waters, the chemical composition of the water has any direct influence upon the fauna. This might be expected in view of the great variation in composition which even the same water may undergo. Such correlations as have been found between faunas and types of fresh water (e.g. hard and soft) might be attributable to the floral food supply which is more influenced by the composition of the water. *Gammarus pulex* can be kept in a solution of pure sodium chloride so long as this is of the order of concentration of fresh water (Beadle & Cragg, unpublished experiments), which suggests that other fresh-water animals may be to some extent independent of the ionic composition of their environment.

Evidence is accumulating that many fresh-water animals, under conditions where the possibility of absorption from the medium or from food is excluded, show remarkable powers of retaining salts. Several have been kept in distilled water for periods which, considering the size of the animals, leave no doubt that the loss of salts to the medium proceeds very slowly, e.g. *Daphnia magna* 10 days (Naumann in Krogh, 1939, p. 96), *Aedes detritus* larvae 10 days (Beadle, 1939), *Gammarus pulex* 8 days, *G. duebeni* (fresh-water variety) 4 days, and *Asellus aquaticus* 8 days (Beadle & Cragg, 1940*c*). *Daphnia*, *Aedes* and the two *Gammarus* species are shown to absorb chloride from low concentrations, but their powers of retention are so well developed that it is doubtful whether, under natural conditions, absorption from the medium would play any significant part. Experiments leave no doubt that it is increased power of salt retention which distinguishes the fresh-water from the brackish variety of *G. duebeni* and which has enabled it to invade fresh water. Of the nature of this retention mechanism we have no knowledge, but it presumably combines very efficient renal reabsorption with a highly impermeable body surface. It is not, however, clear how the latter can be reconciled with absorption of ions from the medium. It is possible that the organs concerned in absorption, which in *Eriocheir* are the gills (Krogh, 1938), can in some species prevent loss of ions in very dilute media by continuing their absorptive action, that is to say by an active rather than a passive barrier to diffusion, a mechanism in fact essentially similar to renal reabsorption.

Some interesting results have been got by adding sea water to the fresh-water medium. The blood concentration curves obtained are shown in Fig. 2. The upper limit in each case is that which can be reached with relatively rapid increase of salinity spread over at most a few weeks. *Eriocheir* can be plunged direct from fresh water into sea water without harm, but this is also true of the purely fresh-water crab *Telphusa* (Duval, 1925), and the factors which apparently confine the latter to fresh waters would be worth investigating. It is strikingly shown in Fig. 2 that the upper limit of tolerance is closely related to the initial blood concentration. This is perhaps not unexpected if it be granted that the tissues of fresh-water animals have become adapted to varying degrees of lowered blood concentration which cannot now be raised by more than a certain amount without fatal results. This explanation was adopted by Beadle & Cragg (1940*a*) from their work on *Gammarus* spp. in which parallel measurements were made of tissue and blood chloride. But it is also noteworthy that the upper limit is reached when the blood concentration approaches that of the medium (Fig. 2), and Schlieper (1933) suggests that

the upper salinity tolerance limit of *Potamobius fluviatilis* (Herrmann, 1931) is determined by its inability to obtain sufficient water from an isotonic medium for the excretion of waste products. Increase of external salinity did in fact progressively reduce the amount of urine formed. Both of these factors may operate. It should be relatively easy to study the tolerance of the tissues to increased concentration of a perfusing medium with the technique used by Wells & Ledingham (1940) on marine polychaete worms, and the effect of increased salinity on the production of urine could be extended to other fresh-water animals. We shall see in the next section that the penetration of highly saline waters by fresh-water animals involved the maintenance of a low blood concentration as well as the adaptation of the excretory organs to function in both iso- and hypertonic media.

There are few data available with which to relate the initial blood concentration and tolerance range (determined in the laboratory) with the extent of penetration into brackish waters by fresh-water species which are unable to regulate in a hypertonic medium. Välikangas (1933) recorded several fresh-water molluscs, including *Limnaea peregra* and *Anodonta* sp., from the Baltic Sea in salinities of 5–6 ‰. *Asellus aquaticus* was found in the Baltic in 6 ‰ (Välikangas, 1933), and, together with *Gammarus pulex*, in the inland saline waters of Westphalia in 25 ‰ (Thienemann, 1913). We should expect from Fig. 2 that *Limnaea* and *Anodonta* in the above situations were living in salinities close to their tolerance limits. The same might be said of *Asellus* if we may judge the probable course of its blood-concentration curve. But we should not expect *Gammarus pulex* to be capable of living in a salinity of 25 ‰.

Acclimatization experiments spread over very long periods have also been tried with fresh-water animals, and some surprising results have been recorded. Beudant (Schlieper, 1933) in 5½ months brought the molluscs *Limnaea*, *Physa*, *Planorbis* and *Ancylus* into sea water. Sexton (1928) succeeded similarly with *Gammarus pulex*, though attempts (unpublished) by myself to repeat this have so far failed, the animals dying when the salinity had reached about 17 ‰ (250 mM.) in 2 months. Plateau (Heilbrunn, 1937, p. 391) in 1 year acclimatized *Asellus aquaticus* to 28 ‰ sea water (420 mM./l.), but this is not much beyond what might be expected from the initial blood-concentration level. The results with the above fresh-water molluscs and with *Gammarus pulex* appear, however, to involve a different form of adaptation. There are two possibilities: (1) that the tissues become acclimatized to a higher blood concentration than they can normally stand and the excretory mechanism is adapted to function when blood and medium are isotonic, or (2) that a regulating mechanism is developed whereby the blood concentration is kept low and thus hypotonic to the medium. This is a possibility which could be easily investigated and, if confirmed, would be of the greatest interest in connexion with the evolution of saline-water animals.

It is possible that temperature may be a factor of some ecological importance for the penetration of marine animals into brackish and fresh waters. Panikkar (1940), commenting on the fact that tropical coasts are the scene of particularly active invasion of brackish waters, attempts to explain this in the light of experimental data. He refers to the observations of Otto (1934, 1937) on the homoiosmotic crabs *Heteropanope tridentata* and *Eriocheir sinensis*, to those of Widmann (1935) on *Gammarus pulex* and *Ligia oceanica*, and to his own on *Leander serratus* and *Palaemonetes varians*, all of which showed a lower blood concentration in summer than in winter. Panikkar also found that when the temperature was raised the blood concentration curves of the above two prawns, when subjected to dilute sea water, were lowered. He suggests that the effect of raised temperature is therefore to lower the optimum blood concentration with the result that there is less strain put upon the osmoregulatory mechanism, and the animals are the more easily able to invade waters of low salinity. This requires experimental confirmation, and it should be mentioned that in unpublished experiments of my own it was found that lowering the temperature had no influence on the final body fluid concentration of *Nereis diversicolor* in dilute sea water, though a considerably longer time was taken to reach equilibrium. Moreover, the survival time in low dilutions was much increased at low temperatures, though it must be admitted that this may have been due to a reduction in the rate of bacterial decomposition in the medium, which was considerable at higher temperatures. But it is surely a *non sequitur* that, because the blood concentration is reduced by raising the temperature, the optimum concentration is thereby lowered. We do not know what constitutes an optimum blood concentration, and in fact all aquatic organisms, and in particular those referred to in this paragraph, remain apparently healthy when the blood concentration fluctuates over a considerable range in response to changes in the medium. It may well be, on the contrary, that a rise of temperature has the effect of reducing the effectiveness of the regulating mechanism so that the blood concentration falls to a level closer to the danger limit for the tissues. Another suggestion previously made by von Martens (see Panikkar, 1940) seems at the moment to be equally reasonable, namely, that the temperature conditions in tropical brackish and fresh waters are more stable and there is less likelihood of a fall to freezing-point, and for this reason they are more easily colonized than those in cooler climates.

V. PENETRATION OF INLAND SALINE WATERS

Inland saline waters are frequently formed in closed drainage basins as in the Caspian region (e.g. Lake

Bulack (Suworow, 1908)), or they may originate from springs, saline at the source from the leaching of salt-bearing rocks, for example in Lorraine (Florentin, 1899), and in French North Africa (Beadle, 1943). The latter occur also in closed basins and are thus further concentrated by evaporation. Coastal salt-marsh pools, which we have classified above as brackish waters, if isolated for a sufficient time from the sea, have the characteristics of 'saline waters'. Compared with sea and fresh waters, saline waters are with difficulty colonized by organisms. The salinity is not only liable to become very high but is subject to great fluctuations according to the inflow of fresh water and the rate of evaporation, and complete desiccation is common. One water may differ greatly from another in composition according to the nature of the source, and the concentration of a given water by evaporation normally results in a radical alteration in the relative proportions of ions owing to differential precipitation (Beadle, 1943). Success in such an environment would appear to involve almost complete independence of the concentration and composition of the medium. To quote some extreme examples: *Artemia fertilis* and ephydrid larvae can flourish in water of 222 °/₀₀ salinity in the Utah Salt Lake (Allee, 1926), and copepods, rotifers and chironomid larvae were found in Lake Bulack in 285 °/₀₀ salinity (Suworow, 1908), and as an example of adaptation to water of unusual composition the mosquito larva *Aedes natronius* and ephydrid larvae were found in an alkaline volcanic crater lake in Uganda of 39 °/₀₀ salinity and 0·7*N* alkalinity (Beadle, 1932).

Attempts to correlate salinity and the nature of the fauna and flora have been made by Florentin (1899) in Lorraine, by Thienemann (1913) in Westfalen, and by Beadle (1943) in Algeria. From these and from the less extensive observations of several other workers (see Hesse, Allee & Schmidt, 1937, pp. 369 ff.) we may draw some general, though to some extent tentative, conclusions. On ecological grounds saline-water animals may be roughly divided into three groups, between which there is considerable overlapping. (1) Those which, though normally inhabitants of fresh waters, are commonly found in saline waters whose salinity does not exceed about 20 °/₀₀. There are a great many such species, many of which are also found in coastal brackish waters, e.g. *Daphnia magna*, the rotifer *Notolca scapha*, *Rana ridibunda* and *Bufo vulgaris*. (2) Those having a distinct preference for salt water, though many are also found in fresh water. Judged, however, from their distribution they are limited to a salinity range not exceeding about 50 °/₀₀. Some coastal brackish water species which have found their way into inland saline waters, are included here, e.g. *Palaemonetes varians*, but most are of fresh water origin, e.g. *Cyclops bicuspidata*, the mollusc *Hydrobia brondeli* and the fish *Cyprinodon fasciatus*. Included in groups (1) and (2) are many insect larvae, especially Diptera. (3) Those animals which are found in the highest salinities, in some cases approaching saturation, e.g. *Artemia salina*, *Actodiaptomus salinus*, the rotifer *Pedalia fennica*, the Diptera *Ephydra macellaria*, *Aedes detritus* and *Anopheles multicolor*. Those species which can withstand the highest salinities have the widest range (Fox, 1927, p. 44), and some are even on occasions found in fresh water (*Anopheles multicolor*) or can survive in fresh water in the laboratory (*Aedes detritus*). As a general rule the higher the salinity of the water the smaller the number of species capable of living in it, and consequently the less are the successful ones hampered by competition. It is well known that in highly saline waters organisms such as *Artemia*, *Pedalia fennica* and certain algae may multiply sufficiently to cloud the water and even to give it a soupy consistency (Allee, 1926; Beadle, 1943).

The above classification, based on field collecting, is provisional and valuable only as a preliminary marshalling of the facts available. Further field work on salinity distribution is required, but, unsupported by experimental work on osmoregulation, it is unlikely to throw much further light upon the mode of evolution of the saline-water fauna. So far only two animals which inhabit highly saline waters have been investigated with a view to discovering the nature of the regulatory mechanism. These are *Artemia salina* and the larva of *Aedes detritus* (Fig. 3). As might be expected, they have great powers of hypotonic regulation, but it is of particular interest that the blood concentration is maintained at a level characteristic of fresh-water animals, which concords with their fresh-water, not marine, origin. *Aedes detritus* larvae are also capable of hypertonic regulation and can thereby live in waters of low salinity and even, in the laboratory, in fresh and distilled water (Beadle, 1939). For comparison, the curve for the fresh-water *Aedes aegypti* is shown in Fig. 3, which has no powers of hypotonic regulation. An example of less well-developed hypotonic regulation is given by the brackish water hemipteran *Sigara lugubris*, whose range is correspondingly limited although greater than that of the fresh-water *S. distincta*. *Artemia salina* has apparently lost the powers of hypertonic regulation possessed by fresh-water phyllopods such as *Chirocephalus* (Panikkar, 1941*b*); it is now confined to highly saline waters.*

Little has as yet been discovered of the nature of the hypotonic regulatory mechanism in these animals. Beadle (1939) found that, though the general body surface of the *Aedes detritus* larva is apparently impermeable to salts and water, of which there is a

* *Artemia salina* will live and breed perfectly well in sea water, but if introduced into an aquarium containing anemones it is rapidly eaten. A reason for its restriction to highly saline waters in nature seems thus to be an inability to defend itself in the sea. Lowndes (1933) explained the restriction of *Chirocephalus diaphanus* to temporary pools on similar lines. (EDITOR.)

considerable exchange through the gut. The experiments indicated that there is probably a mechanism for active salt excretion in the posterior end of the body, possibly in the malpighian tubes. Nothing is known about *Artemia salina*, though Krogh (1939, p. 98), using heavy water as indicator, showed that there is an exchange of water between body fluid and medium, which suggests that active excretion of salts is necessary for the maintenance of a low blood concentration. There is no doubt that compared with most other aquatic animals the body surface of saline-water species is very impermeable, and the strain which otherwise would be put upon the regulatory mechanism is thus considerably relieved, though some exchange must necessarily continue via the gut. It is interesting in this connexion that the anal gills, which in the fresh-water *Aedes aegypti* larvae are large and form the only portion of the body surface permeable to water (Wigglesworth, 1933), are reduced in *Aedes detritus* to vestiges and invested in a thicker cuticle which is probably impermeable (Beadle, 1939).

Experimental work on the effects of altering the relative proportions of the ions in the medium is scanty, but the results indicate that animals adapted to high salinities are not dependent upon a physiologically balanced external medium which is a necessity for most marine animals. Thus Worley (1929) kept the rotifer *Brachionus plicatilis* in calcium-free sea water. The larva of *Aedes detritus* can be reared in distilled water and in a pure sodium chloride solution isotonic with sea water, although in the chlorides of potassium, magnesium and calcium movement stopped in a few hours, but the hypotonic level of the blood was maintained for at least 24 hr. (Beadle, 1939). It is well known that *Artemia salina* can be cultured in pure sodium chloride solutions. On the other hand, it appears that true fresh-water animals are more easily acclimatized to salt water if this is balanced. *Gammarus pulex* can be adapted to higher concentrations of sea water than of pure solutions of its constituent salts (Ostwald in Höber, 1926).

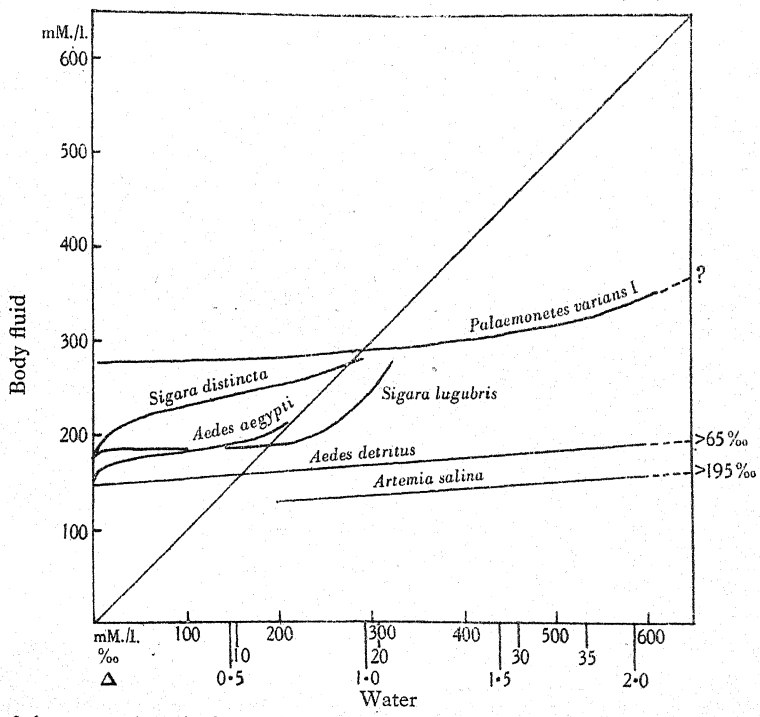

Fig. 3. Relation of the external to the internal medium in animals inhabiting saline waters. *Sigara* and *Aedes* are introduced for comparison. (*Palaemonetes varians* (Panikkar, 1941*a*); *Sigara distincta*, *S. lugubris* (Claus, 1937); *Aedes aegypti* (Wigglesworth, 1938); *A. detritus* (Beadle, 1939); *Artemia salina* (Medwedewa, 1927)).

When we have sufficient experimental data it will no doubt be found more satisfactory to group saline-water animals according to the degree of development of the hypotonic regulatory mechanism. There can be little doubt that most of the animals in group (1) above are incapable of hypotonic regulation, and, by analogy with the fresh-water animals of Fig. 2, we should expect a correlation between the salinity tolerance and blood concentration in fresh water. There would probably be little reason for the retention of the intermediate group (2), whose members no doubt show the capacity for hypotonic regulation in varying degrees, while those in group (3) most certainly have it well developed. The potential value of the *Gammarus* spp. as material for

research is still further increased by the recent discovery of a new species (*G. tigrinus*) in the saline waters at Droitwich (Sexton, 1939). The water was, however, of relatively low salinity (2–3 ‰), and it is possible that this species will show no powers of hypotonic regulation.

Since several brackish water animals of marine origin are capable of hypotonic regulation (Fig. 1), it is curious that the majority of saline forms and all those capable of living in very high salinities are of fresh-water origin.* The few species of marine origin found in inland saline waters (including *Palaemonetes varians*, whose blood is hypotonic in sea water) have not been reported from waters much more saline than sea water (Beadle, 1943). No experiments have apparently been done to find the upper salinity tolerance limit of such animals, but there is obviously another factor of great importance which determines their ability to colonize very saline waters. It has already been mentioned that such waters are particularly liable to complete desiccation, and drought-resistant stages are probably essential for permanent establishment. Such adaptations, usually in the egg although sometimes in later developmental stages (e.g. *Aedes* spp. and some rotifers), are characteristic of fresh-water animals whose environment is much more liable to extremes of physical and chemical conditions than the sea and brackish waters in contact with the sea, the inhabitants of which are mostly without such adaptations. It certainly appears to be true that those inland saline-water animals which are of marine origin (e.g. *Palaemonetes varians*) are confined to waters not subject to complete desiccation. It seems therefore that, though several marine and fresh-water animals have developed a mechanism for hypotonic regulation, the fresh-water forms alone have succeeded in colonizing highly saline inland waters, partly because they are able to resist the rigours of complete drought.

In view of the successful penetration of saline waters by fresh-water animals, it is surprising that so few have returned to the sea. The marine teleost fishes may be of fresh-water origin and the concentration of their blood, like that of inland saline-water animals, is maintained at a level not much above that of their fresh-water relatives (Smith, 1932; Baldwin, 1937). Other examples are rare, though several inland saline-water types (e.g. rotifers and insect larvae) are to be found in coastal salt marshes whose salts are derived from the sea. Here, however, the conditions resemble those in inland saline waters. One of the few notable exceptions is a Samoan chironomid (Buxton, 1926) which lives in the open sea, although in shallow water; not only in the larval and pupal stages but even as the adult, which is wholly aquatic and worm-like in form, the legs and wings having practically disappeared. Buxton (1926) discusses the problem of the inability of insects in general to colonize the sea and is able to reach no definite conclusions. The possible inhibitory factors seem to be the water movements, the predators and the food supply. Most probably it is chiefly water movements which prevent the mosquito *Aedes mariae*, whose larvae are found in sea-water splash pools on the coasts of North Africa, from breeding in the open sea. Whatever the nature of the barriers which have, with certain exceptions, prevented the colonization of the sea by fresh-water animals, there is little doubt that salinity is not an important one.

* Hesse *et al.* (1937, pp. 29, 30 and 167) state that *Nereis diversicolor* has been reported from concentrated sea water, but no details of localities or references are given. Unpublished experiments of mine have shown that in 150% sea water this species can live several days in a very shrunken condition, but its body-fluid concentration follows that of the medium.

SUMMARY

In order to colonize fresh waters, marine animals must maintain a total concentration of the body fluids above that of the external medium. This review is concerned with the probable course by which this independence was achieved during the evolutionary history of fresh-water animals, so far as can be judged from ecological and physiological research. Many marine animals can endure a considerable dilution of the sea water without regulating their body fluids, the tissues being capable of normal function so long as the rate is not too great at which the body-fluid concentration drops. *Nereis diversicolor* shows the beginnings of osmoregulation: a slight body fluid hypertonicity can be maintained when the external medium is dilute, and, when that medium fluctuates, the changes are sufficiently damped before they reach the tissues for the functioning of the latter to be unimpaired. By these means such animals have invaded brackish waters, and penetration of fresh waters may have been accomplished by some other groups through the immediate lowering of the blood concentration and subsequent development of a regulatory mechanism. On the other hand, some Crustacea (*Eriocheir sinensis* and *Telphusa fluviatilis*) have been able to invade brackish water thanks to a different regulating mechanism which prevents much diminution in body-fluid concentration. Active absorption of salt from the medium through some part of the body surface (e.g. the gills) is an important component of this mechanism, though uptake from food via the gut must also contribute. The excretory organs, however, do not play a part, since the urine is isotonic with the blood. Most fresh-water animals have a low blood concentration and the regulatory mechanism is assisted by renal reabsorption. Many are capable of actively absorbing ions from fresh water when the blood is artificially diluted, but it is not certain how far this is an important component of the mechanism in nature. On the other hand, several have remarkable powers of retaining salts, due no doubt to the high impermeability of most of the body surface. But it is possible that the organs concerned in absorption may also, by the same action, prevent loss of salt in very dilute media. Some animals however, are continuously dependent upon food for maintaining the salt level of the blood.

The lower the blood concentration of a fresh-water animal the lower the concentration of brackish water to which it can be adapted. This is no doubt partly due to

irreversible adaptation of the tissues to a dilute body fluid, but the upper salinity tolerance limit may also be determined in part by the inability to obtain enough water for excretion from anything but a hypotonic medium. In the laboratory some marine animals have been adapted to fresh water and some fresh-water species to sea water by altering the concentration of the medium extremely slowly. It seems that under such treatment the extension of the tolerance range is due to the development of a new mechanism not originally functional. Inland saline waters of low concentration have been invaded by some fresh-water animals which cannot maintain their blood hypotonic to the medium, and, where the geographical situation permits, by some brackish water species. Adaptation to highly saline waters, however, requires a mechanism for hypotonic regulation, and all animals living in such waters are of fresh-water origin. They can maintain their blood concentration at a level typical of fresh-water animals. Some brackish water species are also capable of hypotonic regulation, but have not invaded waters of high salinity, probably because, unlike most fresh-water animals, they do not produce stages resistant to the drought which is of common occurrence with inland saline waters.

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